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Over the last four years we conducted and published a radioimaging trial using In-111 MX-DTPA huBrE-3 in patients with metastatic breast cancer. The results suggest that it is possible to administer therapeutic doses of Y-90 MX-DTPA huBrE-3 to patients. We are currently enrolling patients onto therapeutic trials of dose fractionated Y-90 MX-DTPA huBrE-3 to patients with metastatic breast cancer. We have also performed numerous preclinical experiments in the nude mouse human tumor xenograft model. We have demonstrated significant anti-tumor efficacy of combination continuous infusion topotecan and Y-90 MX-DTPA BrE-3. After tumors were allowed to grow for 21 days, the combination of 200uCi of 90Yttrium and 1 mg/m2 for 14 days via Alzet pump demonstrated complete tumor regression in 10 of 13 mice. As demonstrated in subsequent experiments, tumor response was specific to the BrE-3 antibody. This effect persisted with dose fractionation of antibody. In vivo studies demonstrated an increase in apoptosis and a decrease cell proliferation rate which was additive in the presence of topotecan and Y-90 MX-DTPA BrE-3. In our final year we continued these experiments using gemcitabine in a human pancreative cell xenograft nude mouse model and demonstrated signficant activity of combination Y-90 MX-DTPA BrE-3 and gemcitabine.

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C Wasserhet _____ 9/18/98

PI - Signature   **Date**

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Introduction

The following is a final report for Grant No. DAMD17-94-J-4176 entitled, "Radioimmunotherapy of Metastatic Breast Cancer Using Radiolabeled Tumor Specific Antibodies" for the period of September 1, 1994 to August 31, 1998.

The overall goal of the project was to develop effective therapy for breast cancer using radioimmunoconjugates. As noted previously, the humanized version of BrE-3 (huBrE-3) designed at the Cancer Research Fund of Contra Costa by Drs. Joseph Couto and Roberto Ceriani in collaboration with E. Padlan (NIH) has became available to us during the course of this grant award. The purpose of the design was to diminish the immunogenicity of the frameworks while strictly maintaining the antigen binding affinity.

A strategy aimed at increasing the potential efficacy of radioimmunotherapy is by combining this with other agents such as chemotherapy. As noted in my previous progress report, at NIGH, a model drug that we have helped develop is the topoisomerase-1 inhibitor topotecan. Topoisomerase-1 is a nuclear enzyme involved in unwinding of supercoiled DNA and is integrally involved in a host of cell functions including replication and transcription. In the presence of topotecan, there is a stabilization of the complex formed by topoisomerase 1 and DNA, preventing the religation of the DNA strand. Interaction between the stabilized terniary complex and the replication fork is thought to convert single strand breaks into double strand breaks and cause cell death. Topotecan interaction with this enzyme converts topoisomerase-1 into a "cellular poison" and results in progressive cell death.

As previously noted, we conducted a Phase I study utilizing a novel schedule for administration of topotecan, under sponsorship of CTEP. In this study topotecan was given as an ambulatory infusion in low doses, continuously for up to a 21-day duration. We determined the MTD for heavily pretreated patients to be 0.53 mg/m2/day for 21 days, increasing dose intensity by >50% compared to the conventional (daily x 5) administration schedule. We also observed unprecedented activity in a phase I study, including partial remissions in patients with ovarian and breast cancer (previously received 4-5 regimens) and renal cancer (1). We have a number of ongoing clinical trials combining prolonged continuous infusion topotecan with other chemotherapeutic agents such as 5 fluorouracil and liposomal doxorubicin in phase I trials. In addition, we have piloted a phase II study of combined topotecan and cisplatin as first line therapy in patients with ovarian cancer.

While studies of topo-1 inhibitors in combination with radioimmunotherapy have not yet been reported, experimental models with external beam radiation therapy show that the combination of these two modalities enhance cell kill in cell culture and *in vivo* (2-6). It has been postulated that the synergism between the topo-1 inhibitors and ionizing radiation is due to the ability of topo-1 inhibitors to interfere with repair of radiation-induced DNA damage (7). Ionizing radiation sensitizes cells to topo-1 inhibitors by slowing their progression through S-phase, thus, increasing the number of

cells in S-phase (4). The most optimal effects *in vivo* have been seen when the topo-1 inhibitor is given shortly before the irradiation (5), or concurrently with continuous application (8, 9).

While external beam irradiation of loco-regional disease is possible in a disease like primary non-small cell lung cancer, this is a less feasible approach with respect to metastatic breast cancer which may be more widely disseminated. Radioimmunoconjugates provide a vehicle for targeting therapeutic doses of radiation to widely dispersed tumor throughout the body. Similarly, the above principles of synergy will apply to radiation delivered by this method as well as by external beam, but with improved therapeutic index.

As previously reported, we have demonstrated the feasibility of administering topotecan as a continuos infusion in the mouse model. We also demonstrated an antitumor effect. The data generated thus far support the use of combination therapy for future clinical trials. Prior to conducting such a study, a therapeutic trial of the humanized 90Y-Mx-DTPA huBrE-3 is underway as described below.

With regard to our preclinical work, this past year, we have explored other combinations of chemotherapy and radioimmunotherapy. Specifically, gemcitabine is a nucleoside analog that exerts its effect by inhibiting DNA synthesis during S phase and blocking the progression of cells through G1/S phase. Similar to topo-1 inhibitors, gemcitabine is a potent radiation sensitizer. This has been demonstrated in multiple preclinical studies in both cell lines and animal models (11-13). Gemcitabine has demonstrated activity in a variety of tumors including pancreatic carcinoma, non-small cell lung cancer and breast cancer (14-15).

<u>Body</u>

<u>Clinical Work</u>

During the first year of this grant award, huBrE-3 was produced in large quantities by the Cancer Research Fund of Contra Costa. Purification and safety testing was performed. The antibody was also conjugated to MX-DTPA chelate which is identical to the chelate used for the murine antibody. The FDA approved the IND and a phase I clinical trial of 111In-MX-DTPA huBrE-3 was written and IRB approved. Over the next two years, we completed a phase I study using ^{111}In-MX-DTPA huBrE-3 in patients with advanced breast cancer (previously submitted to you.). We have recently published our results in Clinical Cancer Research (10). We studied 7 patients on this protocol. They each received 2 mg of MX-DTPA huBrE-3 labeled with about 5mCi of Indium[111] plus 48 mg of nonconjugated BrE-3 intravenously over one hour. The antibody infusions were well tolerated. No allergic or toxic side effects were observed. One patient complained of a transient strange taste in her mouth. One patient developed grade 3 thrombocytopenia at 9 days after infusion of antibody. She was concurrently receiving external beam radiation to the spine and had extensive

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involvement of the bone marrow with metastatic carcinoma documented by bone marrow biopsy. Serologic analysis revealed no evidence of immunologic platelet destruction and it was felt that the thrombocytopenia was predominantly secondary to the combination of poor bone marrow reserve and external beam radiation and unlikely to be related to toxicity from the ^{111}In MX-DTPA huBrE-3. Patients underwent serial whole body counting, gamma camera imaging, plasma and urine sampling over one week in order to assess pharmacokinetics, radiation dose, tumor localization and pharmacokinetics. We imaged 76% of known bone, liver, and lung lesions (105 sites total) and identified two sites previously unsuspected (lymph node, bone). Blood pharmacokinetics show a longer half-life for the humanized antibody than for the murine. In six patients the $T1/2_{alpha}$ for the humanized antibody averaged 106.5±8.5 hours and the $T1/2_{beta}$ averaged 114.2±39.2 hours. Radiation dose estimates for (using standard MIRD formalism) have been made for normal organ and tumor. Dose estimates to tumors averaged 70± 31.5 rads/mCi administered for ^{90}Y-MX-DTPA huBrE-3 with average marrow dose estimated at 8.4±11.9 rads/mCi administered. Liver radioactivity uptake averaged 19.7 ± 8.8% injected dose at 24 h after infusion, translating into an average radiation absorbed dose 21.1 ± rads/^{90}Y mCI administered. (see appendix 1). Immunogenicity was studied out to 3 months in all the patients on study. Qualitative analysis of serum incubated with either radioiodinated huBrE-3 or Indium-111 labeled hu BrE-3 demonstrates that compared to baseline serum, there are trace amounts of antibody-antibody formation at 5 weeks and 3 months after antibody infusion. Since these anti-humanized antibodies react equally with the murine BrE-3, we believe that "HAHA" represents an idiotypic response.

Thus far, the results suggest that it is possible to administer therapeutic doses of radioimmunoconjugates to patients. The relatively low immunogenicity may allow for repeated administration. Our protocol closed about one year ago and the results of this trial have been published recently (see publications). The results of this trial and the preclinical work was presented (oral presentation and poster) at the Era of Hope Conference in Washington D.C., November, 1997.

Following this protocol, three phase I protocols have been opened (August, 1997). The protocols entail the same procedure and analyses but have slightly different entry criteria. The three protocols are:

• A Phase I/II Study of the Toxicity and Dosimetry of A Humanized Breast-Directed Monoclonal Antibody (BrE-3) Radiolabeled with Indium (^{111}In) and Yttrium (^{90}Y) (H6837) (see Appendix)

The specific aims for this protocol are:

Specific Aim 1: To determine the toxicity profile and maximum tolerated dose of ^{90}Y-labeled huBrE-3 antibody in a Phase I trial using dose fractionation in patients with advanced breast carcinoma.

Specific Aim 2: To examine the biodistribution and pharmacokinetics of both ^{111}In labeled huBrE-3 and ^{90}Y labeled huBrE-3 over multiple administrations in the same patient and the marrow toxicity of the ^{90}Y labeled immunoconjugate.

Specific Aim 3: a) To measure the radiotoxic effect of dose fractionated intravenously administered radioimmunoconjugates directly using hematopoietic progenitor assays.

b) To relate the marrow and bone localization of ^{111}In and ^{90}Y after coadministration of immunoconjugates labeled with each of these radioisotopes in a dose fractionation regimen to the marrow toxicity.

c) To specifically examine the radiation dose to the marrow estimated for an ^{90}Y immunoconjugate using the biodistribution of ^{111}In BrE-3 in relation to the actual effect on marrow as assessed by hematopoietic progenitor assays and peripheral blood counts.

• A Phase I/II Study of the Toxicity and Dosimetry of A Humanized Breast-Directed Monoclonal Antibody (BrE-3) Radiolabeled with Indium (^{111}In) and Yttrium (^{90}Y) in patients with prior exposure to monoclonal antibodies (PRIOR MAB) (H7371)

Specific Aim 1: To determine the toxicity profile and maximum tolerated dose of ^{90}Y-labeled huBrE-3 antibody in a Phase I trial using dose fractionation in patients with advanced breast carcinoma who have had prior exposure to monoclonal antibodies.

Specific Aim 2: To examine the biodistribution and pharmacokinetics of both ^{111}In labeled huBrE-3 and ^{90}Y labeled huBrE-3 over multiple administrations in the same patient and the marrow toxicity of the ^{90}Y labeled immunoconjugate.

Specific Aim 3: a) To measure the radiotoxic effect of dose fractionated intravenously administered radioimmunoconjugates directly using hematopoietic progenitor assays.

b) To relate the marrow and bone localization of ^{111}In and ^{90}Y after coadministration of immunoconjugates labeled with each of these radioisotopes in a dose fractionation regimen to the marrow toxicity.

c) To specifically examine the radiation dose to the marrow estimated for an ^{90}Y immunoconjugate using the biodistribution of ^{111}In BrE-3 in

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relation to the actual effect on marrow as assessed by hematopoietic progenitor assays and peripheral blood counts

• A Phase I/II Study of the Toxicity and Dosimetry of retreatment with a Humanized Breast-Directed Monoclonal Antibody (BrE-3) Radiolabeled with Indium (^{111}In) and Yttrium (^{90}Y) (RETREATMENT) (H7371).

This will be carried out under the following specific aims:

Specific Aim 1: To determine the toxicity profile and maximum tolerated dose of ^{90}Y- labeled huBrE-3 antibody in a Phase I trial using dose fractionation in patients with advanced breast carcinoma who have already received one cycle of dose fractionated ^{90}Y- labeled huBrE-3 antibody therapy. (H7372).

Specific Aim 2: To examine the biodistribution and pharmacokinetics of both ^{111}In labeled huBrE-3 and ^{90}Y labeled huBrE-3 over multiple administrations in the same patient and the marrow toxicity of the ^{90}Y labeled immunoconjugate.

Specific Aim 3: a) To measure the radiotoxic effect of dose fractionated intravenously administered radioimmunoconjugates directly using hematopoietic progenitor assays.

b) To relate the marrow and bone localization of ^{111}In and ^{90}Y after coadministration of immunoconjugates labeled with each of these radioisotopes in a dose fractionation regimen to the marrow toxicity.

c) To specifically examine the radiation dose to the marrow estimated for an ^{90}Y immunoconjugate using the biodistribution of ^{111}In BrE-3 in relation to the actual effect on marrow as assessed by hematopoietic progenitor assays and peripheral blood counts.

The patients eligible for these protocols have the following similar characteristics:

Patients must have histologically confirmed, metastatic or recurrent breast carcinoma which expresses BrE-3 antigen.

Patients must have measurable or evaluable disease.

Karnofsky performance status of ≥ 70 % (ECOG 0, 1, 2)

Patients must have adequate organ function as defined by:

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Neutrophil count ≥ 2500, platelet count > 100,000, hemoglobin > 9 gm/100ml.

Bilirubin < 2.0.

Creatinine ≤ 2.0 or creatinine clearance ≥ 40 ml/ minute.

Normal chest radiograph or pO2 ≥ 80mm Hg on room air and involvement by tumor of ≤25% of pulmonary parenchyma as assessed by CT.

No evidence of active infection which requires antibiotic therapy.

Patients entering this study must be 3 weeks post chemotherapy or radiation therapy and have recovered fully from the toxic effects. Patients may not be on concurrent chemotherapy or radiation therapy. Patients must have failed at least 1 prior standard chemotherapy regimen.

Patients must be at least 18 years of age.

Women of child-bearing potential must have a negative pregnancy test.

Evidence of the BrE-3 antigen on immunohistochemistry using BrE-3 antibody in at least 50% of the tumor cells.

Serum BrE-3 antigen level ≤ 10μg/ml or a total of 25 mg/total plasma volume.

If there is evidence of pulmonary metastases, patients must have adequate pulmonary function (pO2 ≥ 80mm Hg on room air) as measured on standard pulmonary function tests and involvement of ≤25% of pulmonary parenchyma.

Exclusion criteria

Patients with evidence of an active second malignancy are not eligible. Patients with a history of a second malignancy, but no evidence of active disease related to this malignancy, may be considered eligible at the discretion of the investigator.

Clinically significant cardiac disease (New York Heart Association Class III/IV)

Serious infection requiring treatment with antibiotics or other serious concurrent illness.

Concurrent steroid therapy.

Pregnancy or lactation.

Survival expectancy less than 12 weeks.

Active CNS tumor involvement precludes eligibility. This includes spinal cord involvement.

Evidence of extensive skeletal metastases as assessed by bone scintigraphy ; > 25% of the axial skeleton. Bilateral pelvic (sacroiliac) metastases will exclude the patient from eligibility.

Prior bone marrow transplant.

Patients with a history of prior irradiation of >25% of their bone marrow will be excluded.

• In addition for H6837, the following apply:

Inclusion criteria
Patients with no prior exposure to monoclonal antibodies or serum which is non-reactive to huBrE-3.

Exclusion criteria
Prior exposure to monoclonal antibodies and evidence of anti-huBrE-3 antibody.

• For H7371, the following apply (Prior MAb):

Inclusion criteria
Patients who have had prior exposure to monoclonal antibodies but with serum which is non-reactive to huBrE-3.

Exclusion criteria
Evidence of anti-huBrE-3 antibody.

• For H7372, the following apply (Retreatment):

Inclusion criteria
No toxicity related to the previous cycle of radioimmunotherapy >grade II.

Serum which is non-reactive to huBrE-3.

Exclusion criteria
Toxicity associated with previous cycle of Y-90 MX-DTPA huBrE-3 therapy NCI grade II.

Evidence of human anti-humanized BrE-3 antibody in patient's serum.

These proposed treatment studies allow for treatment of up to 18-24 patients. To date one patient has been studied under H6837. Analysis is almost complete.

This patient is a 67 year old woman with breast cancer metastatic to bone and lung previously treated with surgery, external beam radiation therapy to the mastectomy site and axilla, hormones (7/94 - 12/94 and 7/97 - 11/97), chemotherapy (5fluorouracil, adriamycin and cyclophosphamide 12/93-4/94;

Navelbine 9/95 - 11/95, Taxol 12/95- 5/96, Adriamycin and Zinecard 5/97-6/97). Both bony and pulmonary metastases were progressing. She received 2 fractions of Y-90 huBrE-3 antibody (5.9 mCi each). She also received imaging doses of In-111 huBrE-3 antibody. Total doses for each fraction were 50 mg of antibody. Scans showed localization to known measurable disease. She tolerated the administration of the antibody well. There was no evidence clinically of toxicity. Platelet count went from a baseline of 280,000 to 226,000 over the course of six weeks but returned to 274,000 over the subsequent months. No other changes in clinical or routine laboratory indices of toxicity were seen. She had no significant elevation in her HAHA titer over this period of evaluation (week 5). Her final (3-6 month) HAHA is pending.

Although the patient noted a decrease in her bone pain (manubrial metastasis), there was no decrease in size on physical examination. At 7 weeks after initiation of radioimmunotherapy, there was progression of pulmonary and bone metastases.

We hope to enter more patients over the next few months.

Preclinical Work

In the animal model, we performed numerous experiments over the last four years which ultimately demonstrated that Topotecan potentiates the therapeutic effect of radioimmunotherapy using 90Y MX-DTPA BrE-3 monoclonal antibody. In the first year of this grant award, we performed several experiments in the human tumor xenograft model. For example, we tested the utilization of Alzet pumps implanted subcutaneously in mice for the administration of continuous Topotecan over seven days. N/PLOR mice implanted with mammary tumor MGT cells demonstrated a decrease in tumor volume compared to control. We then tested a more prolonged treatment with Topotecan by reinserting another Alzet pump filled with drug on day 7. These experiments were repeated for confirmation several times during year one. Some experiments failed, possibly due to a dose of Topotecan that was too high ($2.4mg/m^2$). During year one, we also performed an experiment using IL-1 in combination with Topotecan. Effects on tumor growth inhibition were not dramatic, although IL-1 did demonstrate a hematologic benefit. We did not pursue continued laboratory experiments with IL-1 because we felt it would be more beneficial to concentrate on combined chemotherapy/radioimmunotherapy in this model (please see Appendix, progress report 1994-1995).

In the fall of 1995, we performed biodistribution experiments of 90-Y MX-DTPA BrE-3 in PLCR female mice. The majority of 90-Y activity was in liver, kidney, and bone at 48 hours post injection (please see Appendix, progress report 1995-1996).

In late 1995, early 1996 we began combination studies in the mouse model. In our first experiment, groups of Swiss nude mice were treated as follows:

Table 1

Group #	Alzet pump	Topotecan dose (per pump)	Y-90
1 (n=3)	none	0	0
2 (n=3)	yes	0	180uCi
3 (n=3)	yes	$2mg/m^2$	0
4 (n=3)	yes	$0.2mg/m^2$	180uCi
5 (n=3)	yes	$0.5mg/m^2$	180uCi
6 (n=3)	yes	$1.0mg/m^2$	180uCi
7 (n=3)	yes	$2mg/m^2$	180uCi

The mice were observed for 3 weeks post therapy. The control mice had continued tumor growth over 21 days, as expected. The groups treated with 90-Y-MX-DTPA-BrE-3 or with topotecan alone had a small decrease in tumor growth relative to control. The groups receiving combination therapy had a much greater decrease in the growth rate of the tumor. The greatest effect was noted in group 6 when topotecan was given at 1.0 mg/m^2 per 7 days. At the higher dose of topotecan (2.0 mg/m2 over 7 days) in combination with 90-Y-BrE-3, all the mice died, presumably from drug toxicity (see appendix, progress report 1995-1996).

In April 1996, we performed another experiment using greater numbers of mice per group. Again, Swiss nude mice were implanted with MX-1 tumors fourteen days prior to starting therapy as described above. For this and all subsequent experiments the dose of topotecan used was 1.0 mg/m^2 over 7 days as this was the most efficacious in the previous experiment. The dose of murine 90-Y-MX-DTPA BrE-3 used in this experiment was between 180-185 uCi in 50 microgram of total protein (0.1cc). This was given as a bolus IP injection on day 0. The mice were randomly assigned to the following groups:

Table 2

Group #	Alzet pump	Topotecan dose(per pump)	Y-90
1 (n=5)	no	0	0
2 (n=7)	yes	$1.0mg/m^2$	0
3 (n=7)	yes	0	180uCi
4 (n=7)	yes	$1.0mg/m^2$	185uCi

As expected, the control group had continued tumor growth. The group of mice treated with 90-Y-BrE-3 or topotecan alone had a decrease rate of growth relative to the control. The combined therapy group had a dramatic decrease in tumor weight which persisted for the duration of the experiment (3 weeks). Thus, we demonstrated in this

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experiment that the tumor burden decreased from baseline in the combined therapy group.

In our next experiment previously reported, on day 21 after implantation of MX-1, mice were randomized into four treatment groups: Control (no treatment), BrE-3 (i.p. 50 ug of murine MX-DTPA BrE-3 labeled with 200uCi of ^{90}Yttrium), topotecan (1mg/m2 for 14 days via s.c. Alzet pump), and combination (BrE-3 and topotecan). Body weights and tumor weights were measured every 3-4 days. As noted in Figure 1, the control mice all died by day 69 after tumor implantation. In the groups treated with BrE-3 or topotecan alone, the mice had reduced tumor growth for about 50 days post treatment but then the tumor grew to sizes comparable to the untreated tumor bearing mice. The mice that received the combination therapy had a substantial decrease in tumor cell growth that resulted in compete tumor regression in 10 of 13 mice. At sacrifice 120 days after treatment, none of the surviving 10 mice had any sign of recurrent tumor.

Our next experiment was performed in order to determine whether the observed tumor response is primarily due to the specificity of the 90Y-MX-DTPA-BrE-3 or to the systemic circulating level of radioactivity given to the animal. A non-specific, isotype matched-matched monoclonal antibody(MOPC) was used for the combined therapy. In addition, the humanized BrE-3 was substituted for the murine antibody as the humanized BrE-3 is used in clinical trial as previously described. Athymic female nude Swiss NIH mice were implanted with human mammary carcinoma (MX-1). On day 21, mice were randomized into one of 5 groups as follows:

Table 3

Group #	hu-90-Y-BrE-3	90Y-MOPC	Topo(1mg/m2 x14days)
1 (n=6)	no	no	no
2 (n=6)	yes	no	no
3 (n=6)	no	yes	no
4 (n=6)	yes	no	yes
5 (n=6)	no	yes	yes

We demonstrated no significant tumor inhibition noted in group #3 and there was no difference in survival between the control group and group #3. The synergistic effect noted with the combined 90Y-MX-DTPA BrE-3 and Topo was not observed in the combined MOPC and topotecan group. Only a transient inhibition of tumor growth was noted in group #5 which was similar in effect and survival to the group treated with BrE-3 alone. The huBrE-3 in combination with topotecan demonstrated the same synergism as with the murine antibody (see Appendix, progress report 1996-1997).

Our next experiment was performed to evaluate treatment efficacy and morbidity in animals treated with a single dose of 200uCi 90Y labeled MX-DTPA BrE-3 compared to two fractionated doses of 90Y labeled MX-DTPA BrE-3 in combination

with topotecan. On day 21 post tumor implantation, mice bearing MX-1 tumors were injected either with a single dose of 200uCi 90Y labeled with MX-DTPA BrE-3 or with 2 weekly injections of 125uCi 90Y labeled with MX-DTPA BrE-3. Mice in each group were then randomized to receive topotecan (1mg/kg for 14 days) via Alzet pump as follows:

Table 4

Group #	200uCI BrE-3 + Topo	125uCi BrE-3x2	125uCiBrE-3x2+Topo
1(n=6)	no	no	no
2(n=6)	yes	no	no
3(n=6)	no	yes	no
4(n=6)	no	yes	no

We demonstrated that both combination groups #2 and #4 had dramatically reduced tumor growth which were of the same magnitude in the fractionated 90Y labeled dosing as in the single dose (see Appendix, progress report 1996-1997). A manuscript of this preclinical work has been submitted to Nature for publication.

Over the last year, in order to determine if this effect was specific to breast tumors, we expanded these preclinical experiments by using combination radioimmunotherapy and chemotherapy in other solid tumors. In addition, we used a different chemotherapeutic agent, Gemcitabine, to assess if this effect was specific to Topotecan.

Initially, we decided to investigate this novel approach for the treatment of pancreatic carcinoma (Panc-1). Gemcitabine has demonstrated effectiveness as a single agent against pancreatic cancer and has clear radiosensitizing activity. This study examined the effect of radioimmunotherapy with gemcitabine on human pancreatic tumor growth in vivo. We used the same radioimmunoconjugate, 90Y MX-DPTA BrE-3 monoclonal antibody that recognizes an epitope of epithelial mucin expressed in many cancers including breast and pancreatic tumors. Nude mice were injected s.c. with human pancreatic carcinoma (Panc-1). On day 28 when tumors were well established, mice were randomized into 4 groups as follows:

Group 1: CTL (no treatment)
Group 2: 90Y-BrE-3 (i.p 200uCi)
Group 3: gemcitabine (120mg/kg every 3 days for 2 weeks)
Group 4: 90Y-BrE-3 and gemcitabine.

Body weights and tumor weights were recorded. Data was expressed as mean/SD, statistical analysis by one-way ANOVA. Groups treated with 90Y-BrE-3 or gemcitabine alone modestly affected tumor growth as compared to untreated control (35mg and 32mg vs. 32mg) at day 60. 90Y-BrE-3 in combination with gemcitabine significantly reduced tumor growth (18mg, $p<0.05$). Our findings suggest that

gemcitabine can enhance the therapeutic index of radioimmunotherapy and reduce tumor growth in human pancreatic tumor xenografts.

In these experiments, toxicity was evaluated as bone marrow progenitor cell viability which was performed 2 weeks after in vivo treatments. Bone marrow cells were obtained sterilely from the animal femur and were plated in methyl cellulose containing media. Colony forming units were determined 3 weeks after plating. Bone marrow toxicities associated with treatments with of 120mg/kg Gemcitabine administered i.p. every 3 days for 2 weeks, 200uCi 90Y BrE3 and the combination were assessed. There is no significant difference in colony-forming units among all three treatment groups as compared to the normal control.

In order to help elucidate the cell-damage mechanism of the combined therapy, several *in vitro* experiments were performed to determine the effect of topotecan, 90Y labeled BrE-3, and the combination on cell proliferation. Two tumor cell lines were utilized for these *in vitro* studies, the MDA-MB 157 (BrE-3 positive) and the MDA-MB 435 (BrE-3 negative) human mammary carcinoma's. Colorimetric (MTT) assay is used to determine cell survival and proliferation. MTT(3-(4,5-dimethylthiazol-2-yi)-2,5 diphenyl tetrasolium bromide) is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria. In this experiment, 1×10^4 cells are plated in 96 wells plate overnight in DMEM media with 10% FBS. 90Y-BrE-3 (5ng to 500ng Ab proteins) are added to half of the wells and incubated for 1 hour. Cells were washed three times to remove all the unbound 90Y-BrE-3 antibodies. Escalating concentrations of topotecan (0uM to 10uM) were then added to the appropriate wells. Cells incubated in media alone served as controls. Plates were incubated for four days and MTT were then added to the wells and allowed to incubate for an additional four hours. Cells were lysed with HCL/isopropanolol and absorbance measured on an ELISA plate reader with a test wavelength of 570 nm and a reference wavelength of 630 nm. BrE-3 binding affinity is ten-fold higher in the MDA-MB 157 than in MDA-MB435. In both cell lines, topotecan exerts a dose escalating reduction of cell proliferation rate (see figure 4). However, in the presence of 90-Y monoclonal Abs, only the MDA-MB 157 cell line which retained 90-Y BrE-3 exhibited a further decrease in cell proliferation rate (data not shown).

In addition, an *in vitro* assay was established with the MDA-MB 157 cell lines and the MDA-MB 435 cell lines described above. Similar to the last experiment, 1×10^4 cells are plated in 96 wells overnight in DMEM media with 10% FBS. 90Y BrE-3 (5 ng to 500 ng Ab proteins) were added to half of the wells and incubated for 1 hour. Cells were then washed three times to remove all the unbound 90Y BrE-3 antibodies. Escalating concentrations of topotecan (0uM to 10uM) were then added to the appropriate wells. At the end of 3 days incubation, cells were lysed and supernatant collected for determining rate of apoptosis. The assay used to measure the degree of apoptosis (Cell Death Detection ELISA, Boehringer Mannheim) is based on the quantitative sandwich-enzyme-immunoassay-principle using a mouse monoclonal antibodies directed against DNA and histones, respectively. This allows the specific

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determination of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. Briefly, anti-histone antibody is fixed absorptively on the wall of the microtiter plate. Supernatant which contained the cytoplasmic fraction of cells were added to the wells and allowed to incubate at room temperature for 90 minutes. After several washings, anti-DNA-peroxidase conjugating solution was added to the wells for an additional 90 minutes incubation at room temperature. Substrate solution was added for photometric analysis and absorbance was measured on an ELISA plate read with a test wavelength of 405nm and a reference wavelength of 490nm. As shown in figure 5 and 6, topotecan is able to induce and increase of apoptosis in a dose escalating manner for both MDA-MB157 and MDA-MB435 cell lines. No additive increase is seen in MDA-MB435 cells treated with 90-Y BrE-3 monoclonal antibody. For the MDA-MB157 cells, however, their is an increased rate of apoptosis in the presence of BrE-3 alone and an additive effect is demonstrated when both topotecan and BrE-3 are administered in combination.

Over the past year, we also attempted to further evaluate the in vitro mechanism of tumor killing of gemcitabine and irradiation using a Cell Survival Assay: After Gemcitabine and/or radiation treatment, cells were assessed for clonogenic survival using a standard assay. Exponentially growing cells were plated at 4×10^5 cells into $25cm^2$ flasks 24 hours prior to experimental treatments. Twenty-four hours after treatment, cells were washed twice with medium and trypsinized, and 10^2 and 10^3 cells were seeded into $25cm^2$ flasks in triplicate. Colonies were allowed to grow for 2 weeks. Cultures were washed with phosphate-buffered saline, fixed with 95% methanol, and stained with 0.04% methylene blue. After counting the numbers of colonies, the results were expressed as survival fraction (calculated by dividing the plating efficiency of treated cells by that of untreated control cells) and cell killing (calculated by subtracting the survival fraction from 1). Gemcitabine exerted a dose-escalating inhibition of cell survival and addition of irradiation was more cytotoxic than Gemcitabine alone (28% increase in inhibition as compared to Gemcitabine alone).

In addition, irradiation, Gemcitabine and the combination were tested for in vitro effect on cell proliferation. The tumor cell lines that we utilized was Panc-1 human pancreatic adenocarcinoma, which expresses the BrE-3 epitope. The colorimetric (MTT, 3-(4,5-dimethylthiazol-2-yi)-2,5diphenyl tetrasolium bromide) assay was used to determine cell proliferation. Briefly, $1x10^4$ cells were plated in 96 wells plate overnight in DMEM media with 10% FBS. Escalating concentrations of Gemcitabine (0uM to 1uM) were then added to the appropriate wells. Half of the plates were irradiated (1Gy) in a cesium irradiator. Cells incubated in media only served as the control. Plates were incubated for three days and MTT was then added and allowed to incubate for an additional four hours. Cells were lysed with HCl/isopropanol and absorbance measured on an ELISA plate reader with a test wavelength of 570nm and a reference wavelength of 630nm.

Gemcitabine exerted a dose-escalating reduction of cell proliferation rate and enhanced the radiation sensitivity of Panc-1 as well (35% decrease as compared to Gemcitabine alone).

Irradiation, Gemcitabine and the combination were also tested for in vitro effect on cell proliferation. Again, the tumor cell lines that we utilized was Panc-1 human pancreatic adenocarcinoma. Briefly, 1×10^4 cells were plated in 96 wells plate overnight in DMEM media with 10% FBS. Escalating concentrations of Gemcitabine (0uM to 1uM) were then added to the appropriate wells; half of the plates were irradiated (1Gy) in a cesium irradiator. Cells incubated in media only served as the control. At the end of 3 days incubation, cells were lysed and the supernatant collected for determining the level of apoptosis using an ELISA (Cell Death Detection ELISA, Boehringer Mannheim, Indianapolis, IN). Gemcitabine is able to induce an increase of apoptosis in a dose-escalating fashion. When both irradiation and Gemcitabine are administered together, their additive effect caused an increase in apoptosis (21% increase as compared to Gemcitabine alone).

Conclusion

In conclusion, over the last year we have completed and published a radioimaging study of ^{111}In-Mx-DTPA-huBrE-3 in patients with metastatic breast cancer. The results suggest that a therapeutic trial of 90-Y-Mx-DTPA huBrE-3 is feasible and repeated doses can be administered. We have opened three therapeutic phase I studies described above. We hope to complete this study within the next year. In addition we have continued to perform our experiments in the mouse model with combination radioimmunotherapy and chemotherapy. As previously noted, the experiments demonstrated that topotecan enhances the therapeutic index of radioimmunotherapy against human mammary carcinoma. This past year, we have performed experiments with the same radiolabeled antibody in combination with gemcitabine in a pancreatic tumor model. Similar to our results in the breast tumors, we have seen the most dramatic response in the mice treated with both gemcitabine and radioimmunotherapy. This data suggests that other drugs with radiosensitizing activity may be substituted for topotecan. In addition, the BrE-3 antibody is not specific to breast cancer expressing this antigen, but may also be active in other epithelial cancers which express BrE-3. Our *in vitro* studies demonstrated a decrease in cell proliferation rate and an increase in the apoptotic rate in the cells treated with gemcitabine. The data generated thus far continue to support the rationale for using this combined modality therapy in women with advanced breast cancer. In the upcoming year we plan to complete our phase I study of fractionated 90Y-BrE-3 and to initiate a phase I study of combined radioimmunotherapy and continuous infusion topotecan in women with advanced breast cancer. In our preclinical experiments, we will investigate other chemotherapeutic agents such as paclitaxel and 9 aminocamptothecan in combination with radioimmunotherapy. If this or other agents reveal synergistic activity we will pursue studying these combinations in patients.

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Appendix

Protocol: A Phase I/II study of the Toxicity and Dosimetry of a humanized Breast-
Directed Monoclonal Antibody (BrE-3) radiolabeled with ^{111}Indium
(^{111}In) and ^{90}Yttrium (^{90}Y)

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Protocol: **A Phase I/II Study of the Toxicity and Dosimetry of A Humanized Breast-Directed Monoclonal Antibody (BrE-3) Radiolabeled with Indium (^{111}In) and Yttrium (^{90}Y)**

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Specific Aims

It is the overall aim of this proposal to use humanized antibodies directed against breast epithelial antigens and an improved understanding of the epitopes targeted by these antibodies to develop effective radioimmunotherapy strategies in patients with metastatic and/or recurrent breast cancer. Two of the most significant obstacles to effective radioimmunotherapy have been the dose limiting radiation toxicity to bone marrow and the immunogenicity of the immunoconjugates used. The work proposed here will be based on a newly gained understanding of the in vivo behavior of humanized radioimmunoconjugates in an attempt to overcome these obstacles. This protocol will capitalize on the expected reduced immunogenicity of the humanized radioimmunoconjugates and employ a dose fractionation approach to attempt to increase the therapeutic index of these radioimmunoconjugates. Our initial efforts will focus on determination of the pharmacokinetics, biodistribution and toxicity of ^{90}Y- labeled huBrE-3 antibody, which based on our recent experience with the murine construct, is expected to show promising in vivo localization in tumors of patients with metastatic breast carcinoma. Clinical Phase I trials will be implemented to evaluate ^{90}Y BrE-3 in a dose fractionation regimen. Although it is expected that dose fractionation may mitigate the marrow toxicity, it is expected that marrow toxicity secondary to radiation exposure will be remain the dose-limiting toxicity. Therefore,we will explore the respective radioisotope content of the marrow and bone as well as the effect on hematopoietic precursors. The NYU Cancer Antibody Trials Group is fully experienced with all aspects of the work proposed, both in preclinical and clinical systems. Extensive, research oriented, clinical services with a long history of performing complex therapeutic protocols, assures high patient accrual rates and careful collection of the necessary data. These studies will be carried out under the following specific aims:

Specific Aim 1: To determine the toxicity profile and maximum tolerated dose of ^{90}Y-labeled huBrE-3 antibody in a Phase I trial using dose fractionation in patients with advanced breast carcinoma.

Specific Aim 2: To examine the biodistribution and pharmacokinetics of both ^{111}In labeled huBrE-3 and ^{90}Y labeled huBrE-3 over multiple administrations in the same patient.and the marrow toxicity of the ^{90}Y labeled immunoconjugate.

Specific Aim 3: To measure the radiotoxic effect of dose fractionated intravenously administered radioimmunoconjugates directly using hematopoietic progenitor assays.

b) To relate the marrow and bone localization of ^{111}In and ^{90}Y after coadministration of immunoconjugates labeled with each of these radioisotopes in a dose fractionation regimen to the marrow toxicity.

c)To specifically examine the radiation dose to the marrow estimated for an ^{90}Y immunoconjugate using the biodistribution of ^{111}In BrE-3 in relation to the actual effect on marrow as assessed by hematopoietic progenitor assays and peripheral blood counts.

A1. Work accomplished:

Breast cancer is the most common cancer in women accounting for 27% of cancers in women. With the recent exception of lung cancer it represents the most common cause of cancer-related death in women. Metastatic breast cancer cannot be cured. Survival beyond the first diagnosis of recurrence averages 2 years (1). Treatment approaches to metastatic and recurrent breast cancer involve multiple therapeutic modalities depending on the nature of the recurrence. While responses to first line therapies including endocrine and chemotherapy may reach as high as 80%, second line therapies are more likely to yield response rates of only 20-35%. Duration of responses is limited. The prevalence of the disease and its refractory nature provide a strong rationale for the development of new therapeutic approaches to metastatic and recurrent breast cancer. Targeting of radioactivity using immunoconjugates directed against breast carcinoma offers a potentially effective therapeutic tool.

A1.1 Radiolabeled tumor specific antibodies

Targeting of radioactivity to tumors using anti-tumor antibodies is evolving from a research endeavor toward a practical diagnostic and therapeutic technique which promises widespread benefits for many common human cancers. Early workers including Pressman et al (2, 3, 4) demonstrated the localization of radiolabeled tumor-specific antibodies in animal and human tumors . Day (5) , Bale, Spar (6) , Mach (7, 8) and Goldenberg (9) showed that radiolabeled polyclonal antibodies could localize in adenocarcinomas. The development of the hybridoma technique of Kohler and Milstein (10) for producing monoclonal antibody advanced the field significantly. Mach first demonstrated (11) that such antibodies could target human tumors. Clinical radioimmunodetection trials which report detection rates of up to 89% (12, 13) suggest that our ability to target tumors effectively with radiolabeled tumor -directed antibodies has achieved a level of success which justifies efforts to deliver therapeutic levels of radiation using this approach . Indeed, recent trials using radioiodinated antibodies in B cell lymphomas (14) support the concept that even when other therapeutic modalities have failed, radiolabeled antibodies may induce responses. To date, preliminary trials of immunoguided radiotherapy in solid tumors have met with limited results . However, studies in this area are still in their infancy. An early therapy trial of I-131 labeled antibody specific for melanoma in two patients resulted in a partial response in 1 (15) . Ongoing trials of I-131 labeled 3F8 antibody in advanced neuroblastoma have yielded partial responses (16) . ^{90}Y polyclonal antibodies directed against ferritin have been used in Hodgkin's disease with 4 partial responses and 4 complete responses out of 11 patients (17) . More recently, I-131 labeled L-6 antibody administration has resulted in partial responses in 4 patients with advanced metastatic breast cancer. Three of these patients developed an antibody response to L-6 (18) .

A. 1.1.1 Radiation toxicity of radioimmunoconjugates

In the radioimmunotherapy of solid tumors several obstacles to successful therapy have been difficult to surmount. The delivery of therapeutic doses of radiation to tumor has been hampered by the radiation toxicity associated with administration of therapeutically effective doses of radioactivity. Since the first radiation toxicity seen in patient's receiving radioimmunotherapy is hematologic, one common solution has been to use bone marrow support strategies to diminish hematologic toxicity including cytokine support (19) and/or autologous bone marrow support((14) . Although it has been suggested that faster clearing immunoreactive proteins, e.g. antibody fragments will reduce the nonspecific irradiation to marrow, this approach also leads to less irradiation of tumor (20) . The use of an anti-antibody to clear unlocalized tumor-specific antibody has also been suggested, but especially with radiometal labeled antibody may lead to unacceptable kidney and/ or

liver doses (20) . Another approach has been to institute dose fractionation regimens (20, 21, 22, 23, 24, 25) which employ lower doses of radioactivity at each individual administration. Animal studies with [131]labeled CEA specific antibody has shown some advantage of a dose fractionation schema in reduced marrow toxicity with comparatively greater tumor response (20) . Possibly, continuous low dose irradiation as might be delivered by more closely spaced serial doses of radiolabeled immunoconjugate may result in radiosensitization of tumor (26) . Initial studies using fractionated dosed of [131]Iodinated B72.3 antibody have shown a trend toward lower toxicity compared to the nonfractionated dose; however, statistical significance has not been shown (21)

A.1.1. 2. Immunogenicity of radioimmunoconjugates

The immunogenicity of murine immunoconjugates has hindered efforts at both dose fractionation regimens and repeat therapy when initial responses are seen (21, 27, 28, 29) .Several approaches have been taken to diminishing human anti-antibody responses to immunoconjugate administration. Immunosuppression with drugs such as cyclosporin A (30) . Others have used plasmaphoresis to reduce serum anti-antibody levels once an antibody response has been mounted (27) .

Still another approach has been to attempt to reduce the immunogenicity of the immunoconjugate itself. The incidence of HAMA after administration of antibody fragments is considerably reduced in comparison to after whole murine antibody . Unfortunately, antibody fragments are considered inadequate for delivery of radioimmunotherapy because of lower uptake in tumor (20) . Recombinant DNA technology applied to monoclonal antibodies has been undertaken to reduce the immunogenicity of whole antibody. Chimerization of murine antibodies replacing the constant region of the antibody with a human construct has been expected to reduce the immunogenicity. Some studies with chimeric antibodies have shown reduced immunogenicity (31) , but in other clinical studies with chimeric antibody the antibody response has been prominent . A major portion of the anti-antibody response has been against the mouse variable region of the mouse/human chimeric (31) . The anti-isotype response has been less prominent. Further humanization of the variable portion of the protein might diminish the anti-idiotypic response, but also may lead to decreased antibody affinity for the antigen. Recently, humanization of both the constant and variable portion of the BrE-3 antibody has been accomplished with preservation of the CDR's of the antibody in their murine form, maintaining the affinity of the antibody ((32)).

A1.2 Antibodies which recognize breast carcinoma associated antigens

In the study of radioimmunotargeting of breast cancer, several monoclonal antibodies against human breast cancer antigens have been produced. TAG-72, a tumor associated glycoprotein, has been shown by immunohistochemistry to be expressed on breast carcinomas and in vivo studies have shown successful localization in primary tumors, but not lymph nodes (33) . Intravenously administered antibodies specific for CEA have localized 76% of metastases. Previous trials of antibodies specific for human milk fat globule membrane associated antigen have shown limited results (34, 35) .

Another group of antibodies which react strongly to human breast cancers and recognize human milk fat globule (HMFG) antigens include the BrE-3, MC-5 and KC4G antibodies (36, 37) . BrE-3 and Mc-5 antibodies have been developed by Dr. Roberto Ceriani and his colleagues at the John Muir Cancer Center. BrE-3 is available from Coulter Immunology in both the nonconjugated form and as the methyl benzyl DTPA conjugate. BrE-3 is an IgG_1 murine monoclonal antibody which like MC-5 and KC4G recognizes a 400 kD epitope of HMFG. MC-5 and KC4G have undergone Phase I trials in patients

with breast carcinoma (38) . Targeting of tumor by MC-5 was significantly hindered by high circulating levels of the antigen (39) .

Circulating levels of the antigen recognized by BrE-3 are only 5-15% of those recognized by MC-5. The expression of BrE-3 on human breast tumor cells is close to 100% as determined by flow cytometry of a variety of breast tumors grown in immunodeficient mice. In addition, there is little binding of BrE-3 to normal human tissues as determined by studies on formalin-fixed paraffin embedded tissue blocks. Immunohistochemical screening of breast tumor tissue for BrE-3 antigen expression has been positive in all 27 patients evaluated to date in our institution although intensity of staining varies.

For this reason a Phase I trial of In-111 labeled BrE-3 was undertaken in our institution. A total of 15 women with recurrent or metastatic breast carcinoma in a dose ranging study have been studied (9 at NYU Medical Center and 6 at U.C. Davis) (40) . All patients received 5 mCi of In-111 labeled MX-DTPA conjugated BrE-3 in combination with unlabeled, nonconjugated BrE-3 antibody to total 10 mg, 50 mg, or 100 mg. 5 patients were studied at each dose level. Patients were imaged up to 8 days after infusion. We have studied an additional patient to date as part of a ^{90}Y MX-DTPA BrE-3 therapy protocol. Coinfusion of ^{111}In MX-DTPA BrE-3 permitted imaging of this patient as well. 86% of known lesions in these 16 patients were detected by scan. Bone, lung and lymph node metastases were seen except when obscured by liver activity. Two of five liver metastases showed increased accumulation. Assuming that tumor localization of ^{111}In-labeled BrE-3 is comparable to that of ^{90}Y labeled antibody, we estimate that radiation doses to tumor for an ^{90}Y labeled BrE-3 dose might reach as high as 590 rads/mCi. Dose estimates to marrow for an ^{90}Y labeled BrE-3 average 2.1 rads/ mCi assuming that 25% of the marrow volume is blood and that this represents the only source of radioactivity for marrow dose from marrow. Estimates from region of interest analysis were approximately 4-5 times higher. This preliminary data suggests that ^{90}Y labeled BrE-3 may have potential as a radioimmunotherapeutic agent in patients with metastatic or recurrent breast carcinoma. On the other hand, an IgG HAMA response was identified in 7 of 16 patients who received BrE-3 antibody under these protocols making readministration of murine BrE-3 antibody difficult. HAMA responses occurred at all dose levels.

A1.3 ^{90}Ytrium as a radiotherapeutic agent in radioimmunotherapy

Most commonly , ^{131}Iodine has been used as the radioactive moiety for radioimmunotherapy. Dehalogenation in vivo both before the antibody localizes within the tumor and afterwards, release from tumor cells (41) , serves to diminish the effectiveness of an ^{131}I radiolabel. Furthermore, the energetic gamma emission of 131I increases radiation safety concerns: there are greater exposure risks for health care personnel and others who come into contact with patients. Also, hospitalization of the patients is usually required. Because of the energetic gamma emissions of ^{131}I , greater radiation exposure to normal organs results.

More recently, ^{90}Yttrium labelled antibodies have been employed for radioimmunotherapy of solid tumors. ^{90}Y offers several potential advantages over ^{131}I. ^{90}Y is better retained by tumor cells than ^{131}I after the administration of radioimmunoconjugates (20, 41) . The absence of a gamma emission results in decreased exposure to distant organs and also to health care personnel . The energetic beta emission which has an estimated range of ~5 mm offers advantages in solid tumors where

heterogeneity of antigen expression/distribution may exist. With this range of energy deposition, cells in the vicinity of the radionuclide which do not bind antibody may still be irradiated.

Radioimmunotherapy with BrE-3 in nude mice carrying MX-1 tumors has been performed using ^{131}I labeled BrE-3 with a 90.2 % inhibition of tumor growth compared to controls at 28 days. A study of ^{90}Y chelate conjugate of BrE-3 in a similar animal model showed eradication of tumors at 20 days in comparison to control tumors which had increased over 10-fold in volume. These results support the selection of ^{90}Y as the radioactive moiety in the development of this group of radioactive immunoconjugates as therapeutic agents.

The instability of the label is a recognized problem with the DTPA cyclic anhydride chelate and [90]Y (20, 42, 43). In a trial of intraperitoneally administered ^{90}Y, the estimated % injected dose in bone varied from 2-49% with 2-13% in bone marrow (44). Other chelates including the macrocyclic chelates (45) and bifunctional chelates developed by Gansow, such as p-NH$_2$-Bz-DTPA and SCN Bz-DTPA and their methyl derivatives (46, 47) show greater stability in serum and higher tumor to bone ratios in animals (48, 49). We propose to use the SCN-Bz-Mx-DTPA chelate because biodistribution studies in mice using ^{90}Y-SCN-Bz-Mx-DTPA chelate BrE-3 have shown high stability (50). Others have had similar experience with this chelate with other antibodies and [90]Y (Thomas Griffin, University of Massachusetts).

A1.4 Use of ^{111}In labeled immunoconjugates to trace the biodistribution of ^{90}Y immunoconjugates and predict dosimetry

Variation among patients in tumor localization of ^{90}Y labeled antibodies (51) as well as bone and bone marrow accumulation occurs (45). Tumor localization may relate to antigen expression, tumor vascular supply and permeability (51). Marrow and bone localization will relate to loss of radiolabel from the antibody either due to instability of the chelate or to metabolism of the radioimmunoconjugate. Because of this variability, it is important to assess the biodistribution of administered ^{90}Y radioimmunoconjugates in order to predict possible radiotoxicity. Imaging of the radioimmunoconjugate would provide information concerning tumor localization, which may indicate the potential efficacy, and nonspecific localization, (e.g.,skeletal, marrow, and liver), to indicate potential toxicities. However, because ^{90}Y emits a single high energy Beta (2.2 MeV), conventional gamma camera imaging to assess biodistribution cannot be performed. Recently, gamma camera imaging of ^{90}Y using the Bremsstrahlung emissions has been accomplished (52). Preliminary work using this approach to image ^{90}Y immunoconjugates at the University of Massachusetts involves the use of specialized collimators and filters. The feasibility and quantitative nature of these measurements are under investigation (Thomas Griffin, personal communication). An alternative strategy might be to administer a tracer dose of ^{90}Y immunoconjugate prior to the therapeutic dose in order to examine blood pharmacokinetics in individual patients.

Another approach is to use an imageable radioconjugate, like an ^{111}In chelate, to predict the biodistribution of an identically chelated antibody but labeled with [90]Yttrium. The use of ^{111}In for this purpose offers an attractive solution. We recognize that there are differences in the metabolism, stability and biodistribution of ^{111}In-immunoconjugate compared to ^{90}Y-immunoconjugate. [90]Y is less stably bound to some chelates than [111]In. [111]Indium localizes primarily in bone marrow; ^{90}Y localizes mainly in bone

mineral matrix . Preinfusion of an ^{111}In chelate conjugated antibody in a radioimmunotherapy trial with ^{90}Y labeled polyclonal antibodies has been performed (51) . In a comparison of ^{111}In and [88]Y isothiocyanate benzyl methyl DTPA labeled antibody in nude mice, Sharkey found similar concentrations of the two radioisotopes in bone at early times after injection (20) . However, at day 8, [88]Y uptake in bone exceeded [111]In. This may have important implications regarding our ability to use ^{111}In as a tracer for therapeutic planning. Preliminary data from Dr. Ceriani's laboratory in tumor bearing mice using this antibody and the MX chelate for [111]Indium and [90]Yttrium show differences in bone and bone marrow uptake for the two radionuclides particularly at later time points. At early time points in mice (Day 1) the ratio of %ID/g ^{90}Y/^{111}In in bone is approximately 2.2 while at Day 8 this ratio is 22. For marrow the ratio on Day 1 is 1.1 and on Day 8 the ratio is greater than 15.

These differences may represent differences in loss of the radioisotope from the antibody due either to instability of the radiometal bond with the chelate or to metabolism of the radiolabeled chelate immunoconjugate, as well as differences in the physiologic handling of the free radionuclides. The stability of the chelate may also influence accumulation in the liver for these two radionuclides. The effect of these differences on marrow toxicity have not been explored in detail.
We intend to examine the pharmacokinetics of the two radionuclides in each patients a two separate antibody administrations, the first and third of a dose fractionation regimen . We will correlate accumulation of the radionuclides in marrow and bone using bone marrow biopsies at the end of the third treatment. Marrow obtained at this time will be assessed for toxic effects using hematopoietic progenitor assays. We will also examine the immunogenicity of this humanized radioimmunoconjugate during the later phases of the dose fractionation cycle (i.e., at day 7 or beyond) and over the course of clinical follow-up. When possible, we also will determine the radiolocalization in tumor by biopsy. This will provide the basis for assessing the toxicity of dose fractionated radioimmunotherapy, the immunogenicity of humanized antibodies used in this schema, ^{111}In biodistribution in relation to ^{90}Y biodistribution, and for developing an improved means of estimating ^{90}Y immunoconjugate dosimetry to the marrow.

A1.7 Rationale for specific aims:
    The NYU group has a strong interest in the conduct of clinical trials on the use of radioconjugated monoclonal antibody-based strategies in the therapy of breast cancer. Based on recent experience with murine BrE-3 monoclonal antibody: the excellent tumor localization achieved with this immunoconjugate and the favorable radiation dose estimates as well as th econsiderable immunogenicity of the murine construct, we have chosen to direct our efforts towards defining the distribution, pharmacology and toxicity of dose fractionated ^{90}Y-humanized BrE-3 radioconjugate by directly measuring radioisotope content of blood, marrow and bone and monitoring marrow response, measuring antibody formation and looking at the effect of any immunogenicity on the ability to target tumor over three dose fractions.
    Thus, we have completed a Phase I study of ^{111}In-murine BrE-3, which defined the tumor localization, general toxicities and distribution of the antibody. With completion of the ^{111}In-muBrE-3 trial, we have initiated a similar Phase I study of ^{90}Y-muBrE-3 . The protocol will include use of a combination of the ^{90}Y conjugate with a tracer dose of ^{111}In-BrE-3 to permit imaging. We will use data generated from this study to examine dosimetry and begin to develop a basis for therapy planning in future trials of ^{90}Y radioconjugates (Specific Aim 2). This will depend on relating the biodistributions of two

chemically similar but not identical radioisotopes used to label the same conjugate. We anticipate that although the biodistribution of ^{111}In and ^{90}Y will not be the same, there will be a predictable and reproducible relationship between them. The biodistributions and conventional estimates of radiation dose based on the MIRD formalism can be correlated with the measured radiotoxicity of ^{90}Y BrE-3 on marrow as assessed by peripheral blood counts and , more specifically, by assays of marrow precursors. This will be the first true analysis on a cellular basis of the toxicity of radioimmunotherapy on marrow precursors. Finally, we will use cytokines with marrow protective effects to attempt to increase the therapeutic index of 90Y-BrE-3 (Specific Aim 3). We will focus on IL-1 initially because of its potential enhancement of hematopoietic precursors and direct effects on tumor vascular permeability, but other cytokines such as GMCSF or sequential IL3 and GMCSF which activate both early and late precursors may be explored. We believe that this stepwise and systematic approach to our objective will provide a rigorous and efficacious test of the hypothesis that such radioconjugates will be useful in the therapy of breast cancers.

A2.Work accomplished at NYU

The NYU Cancer Antibody Trials Group combines the expertise of nuclear medicine physicians, medical oncologists, pharmacologists, pathologists, and basic scientists, all with fundamental interest in application of monoclonal antibodies to cancer to accomplish such studies. This multidisciplinary group meets on a regular basis for review of protocols, coordination of projects and discussion of data. The group further serves as a nucleus for teaching conferences related to immunodiagnosis and therapy. The Antibody Trials Group has drawn directly on the resources of the NYU Cancer Center in the course of its work.

The Cancer Center resources draw on the patient resources of active clinical oncologic practices at NYU Medical Center, Bellevue Hospital Center and the Manhattan Veterans Administration Medical Center. For 1989 an estimated total of 7600 new and follow-up cancer patients were treated at these 3 institutions. Of these, 3144 were newly diagnosed cases including 282 new cases of breast cancer. This patient population has supported patient accrual efforts in a number of breast cancer related trials. The clinical members of the NYU Cancer Center have consistently demonstrated a commitment to enrolling patients in clinical investigations in breast carcinoma. Ongoing trials in breast cancer include continuation of the randomized trial of FAC with and without ICRF-187, and an additional 11 chemo/hormonal therapeutic trials related to breast cancer. Accrual of metastatic breast cancer patients to a clinical trial has averaged 2-3 patients/ month in the past year; there should be no difficulty meeting accrual goals for the studies in this program.

The NYU Cancer Center has a long history of cooperation in performing clinical therapeutic trials in breast cancer and clinical trials of monoclonal antibodies, conjugated and nonconjugated. This work has been accomplished through the joint efforts of Medical Oncology, Surgical Oncology, Nuclear Medicine, Diagnostic Radiology, Radiation Oncology and the Oncologic Pharmacology Laboratory. As a result of this activity, well - established lines of communication have been developed. These are formalized in weekly work- and protocol-oriented conferences, and maintained through the organization and the support cores of the Kaplan Cancer Center. The Kaplan Cancer Center has had a very active multidisciplinary collaborative effort in the treatment of breast cancer. Over the last years we have had significant contributions to the literature in this area. An example is the study of the role of cardiac protection, including exploring a Phase II trial of infusional adriamycin with ICRF-187 as a method of reducing cardiac toxicity, and a major Phase III

study recently published demonstrating cardiac protection with ICRF-187 (53) . A total of 150 patients with metastatic breast cancer were entered into this trial .

We are also active participants in the Eastern Cooperative Oncology Group clinical trials. Included in this are adjuvant and metastatic disease protocols. This ECOG link is important for potential technology transfer should significant antitumor activity be demonstrated from this research.

The Oncopharmacology Laboratory under the supervision of Dr. Leonard Liebes is a major resource available to the Antibody Group. The laboratory has extensive experience in pharmacokinetic studies of anti-cancer agents. A particular emphasis has been the study of the new agent ICRF-187 when used for cardioprotection from anthracycline toxicity. This laboratory has defined the pharmacokinetics of this agent in the course of a series of clinical trials at NYU. This data has been integral to the submission of an NDA for this drug. The laboratory has been performing pharmacokinetic studies for antibody trials at NYU including the Anti-B4 blocked ricin trial. This Laboratory has been responsible for the pharmacokinetic analysis of 111In BrE-3 during its initial clinical trials performed at NYU.

A Phase I trial of ^{111}In BrE-3 antibody in patients with metastatic and recurrent breast carcinoma has been completed recently at NYU through the cooperative efforts of the NYU group and the John Muir Cancer Center. In addition,the protocol for a Phase I therapeutic trial of ^{90}YBrE-3 antibody in patients with metastatic and recurrent breast carcinoma has received institutional approval.

A2.1 Biologic Clinical Trials Experience

The Division of Oncology at NYU has extensive experience in the design and execution of early clinical trials of biologics and combined biologics and chemotherapeutics. Many of these trials have been accompanied by laboratory investigations performed in The Core Biologic Response Modifier Laboratory. One of the functions of this laboratory, which is a shared resource of The NYU Cancer Center, is to perform correlative laboratory studies for clinical trials. Over the past several years the laboratory's work has focused on the effects of biologics as modulators of monocyte and lymphocyte cytotoxic activity. Interferons, GMCSF, MCSF, IL-2, IL-4, CGP 19835a (muramyl tripeptide in liposomes) and SDZ 62-826 (an alkylphospholipid compound) have been studied. NYU holds a NCI biologics contract from the BRMP for the testing of new biologics with Dr. Ronald Blum as Principal Investigator. Under this contract we have recently completed an NCI supported Phase I b trial of GMCSF designed to examine in vivo effects on monocyte activation patients. Twenty four patients were treated on this study at varying dose levels of GMCSF. Patient monocytes studied at multiple time points for in vitro cytotoxic activity against HT29 (Human Colon Carcinoma) target cell line showed enhancement of monocyte cytotoxicity after in vivo GMCSF administration (54) . We have performed a series of trials of combined biologics and chemotherapeutic agents, including a Phase I trial of alpha interferon and cisplatinum followed by Phase II trials of this combination. In the area of the use of biologics for bone marrow protection we originally studied GMCSF and are now moving on to other agents and combinations. Under NCI auspices we performed a Phase I trial of escalating doses of doxorubicin accompanied by ICRF-187 for cardioprotection and GMCSF for bone marrow protection. Our goal was to develop a dose intense regimen that could be given in the outpatient setting with acceptable toxicity. Seventeen patients were treated on this study (53) . Because unsatisfactory escalation of doxorubicin was achieved with GMCSF alone as the marrow protector we have submitted a LOI to begin studies of IL-3 plus GMCSF when NCI drug supply is available. As part of the AIDS Clinical Trials Group, NYU was the principal site for a Phase I study of the combination of mBACOD plus GMCSF in the treatment of HIV associated lymphoma (55) . We are performing currently a Phase I trial of IL-3 as a bone marrow protector with the combination of cytoxan and carboplatinum in the treatment of

ovarian cancer. This study will determine which of two schedules of IL-3 administration has the most beneficial effect on hematopoietic recovery.

A2.1   Monoclonal Antibody Experience

Through the Eastern Cooperative Oncology Group we participated in a Phase II study of unconjugated 17-1A monoclonal antibody in the treatment of advanced pancreatic carcinoma. Antibody (500 mg) was given intravenously three times weekly for eight weeks. Among the 28 patients there was an 18% incidence of hypersensitivity reactions which were easily managed. This study demonstrated feasibility of repetitive antibody dosing with this unconjugated antibody. Pretreatment and post treatment levels of circulating 17-1A did not change significantly during the eight weeks of treatment. However, no objective responses were seen.

We have performed several studies with anti melanoma antibody-ricin A chain immunoconjugate XMMME-001-RTA (Xoma). Our initial studies with this immunoconjugate demonstrated that durable partial remissions could be seen with this therapy. Essentially all patients developed a host antibody response against both murine immunoglobulin and ricin A chain. At NYU we conducted a subsequent protocol which administered an intravenous dose of 1000 mg/m^2 of cyclosphosphamide shortly after immunotoxin administration. Twenty patients were treated on this protocol. The addition of cyclophosphamide did not have a significant effect on the incidence of antibody formation, but a response rate of 20% was seen with this combination (56).

Also, we have begun a study combining GMCSF with the monoclonal antibody R24 in the treatment of patients with advanced melanoma. This study will involve monitoring of in vitro ADCC activity of patients monocytes and granulocytes. Another Phase I trial begun recently uses an Anti B4 blocked ricin in HIV associated lymphoma. We anticipate studying 20-24 patients in this trial.

A2.2   Previous work in radiolabeled immunoconjugates:

For the past several years the Division of Nuclear Medicine has been engaged in a series of clinical trials to evaluate the efficacy of radiolabeled antibodies in diagnosis and staging of solid tumors. This has been accomplished with the cooperative efforts of Diagnostic Radiology, Medical Oncology, Surgical Oncology and Pathology. Our focus in radioimmunodiagnosis has been the application of single photon emission computed tomography (SPECT) to the imaging of radiolabeled antibodies. In a Phase II/III trial of an ^{111}In labeled anti- CEA monoclonal antibody (^{111}In ZCE025, Hybritech, Inc.) in patients with suspected colorectal carcinoma we found a higher detection rate with SPECT (84%) than planar imaging(61%) (57). In recognizing some of the problems in radiolabeled antibody imaging, i.e., absence of anatomic landmarks, persistent blood pool and other nonspecific uptake, and to improve our understanding of the information available in radiolabeled antibody images, we developed a method of "fusing" or registering SPECT images from radiolabeled antibody images with CT and MRI of the abdomen . This permitted precise matching of tomographic images from the different imaging modalities. This was based on a software package called *qsh* developed by Dr. Marilyn Noz in the Department of Radiology at New York University and Dr. Gerald Q. Maguire from Columbia University (58, 59). Although this fusion algorithm had been used in correlating transaxial brain images and planar images, This was its first application in the abdomen and pelvis (60). We have also extended this algorithm to the chest in the context of an imaging trial in patients with nonsmall cell lung carcinoma using a Tc-99m labeled Fab fragment which recognizes a pancarcinoma antigen (Tc-99m NR-LU-10, NeoRx Corporation). This was a Phase II trial carried out under the supervision of Dr. Kramer at Memorial Sloan-Kettering Cancer Center, now at NYU. Using *qsh* to fuse SPECT images of the chest with CT of the chest, abnormal foci of antibody uptake in the

mediastinum could be anatomically localized to specific nodal groups for more accurate staging of mediastinal disease (61) . Dr. Henry Rusinek in the Department of Radiology has begun to extend the application of fusion using the matched CT or MRI for edge detection for improved scatter correction of the SPECT image (62) . It is anticipated that more accurate SPECT reconstructions performed with this algorithm will be useful eventually for dosimetry calculations. This same software has been applied to the processing of serial planar images obtained in the current Phase I ^{111}In- BrE-3 trial for attenuation correction and region of interest analysis to provide biodistribution data and radiation dosimetry estimates (63) .

As Clinical Director of Nuclear Medicine at Memorial Sloan-Kettering Cancer Center between February, 1989 and June 1990, Dr. Kramer was responsible for supervising and coordinating the Nuclear Medicine efforts in several radiolabeled monoclonal antibody trials. These included a Phase I therapy trial of I-131 labeled OKB7 monoclonal antibody directed against the Epstein-Barr virus receptor in patients with CLL and non-Hodgkin's lymphoma (64) , a Phase I therapy trial of I-131 labeled M195 monoclonal antibody in acute leukemia, an imaging trial of an In-111 anti-CEA antibody in metastatic colorectal carcinoma , and a Phase II/III pre-surgical imaging trial of Tc-99m NR-LU-10 , directed against a pan carcinoma antigen, in nonsmall cell lung carcinoma.

At NYU we have also performed a Phase I/II imaging trial using a cell culture produced ^{111}In ZCE025 in patients with colorectal carcinoma , have completed recently another imaging trial of ascites produced ^{111}In ZCE025 in patients with colorectal carcinoma and completed a Phase I study of 99mTc-labeled CEA specific antibody fragment in patients with lung carcinoma (65) .

A2.3 Present work specifically related to radiolabeled immunoconjugates targeting breast carcinoma.

More specifically related to the proposed work is the recently completed Phase I trial of In-111 methyl benzyl DTPA murine BrE-3 at NYU Medical Center in cooperation with Dr. Ceriani's group at John Muir Cancer.Center and with Coulter Immunology. The blood clearance, the delivery of antibody to tumor , and in vivo stability in humans for radiometal chelate conjugated BrE-3 have been investigated in this recent Phase I trial of ^{111}In methyl benzyl DTPA BrE-3. This trial with ^{111}In methyl benzyl DTPA BrE-3 was designed primarily to assess the toxicity and localization potential of the radiolabeled antibody and to examine the dose (mg) of antibody which will give optimal localization of antibody in tumor as assessed by imaging. A total of 15 women with metastatic breast carcinoma. have been studied: 9 at NYU Medical Center and 6 at U.C.Davis under the supervision of Dr. Sally DeNardo All patients' tumors expressed the BrE-3 antigen as assessed by immunohistochemical staining of previously obtained tissue. In 27 patients (45 tissue samples) screened at NYU, more than 60% of cells stained positive for the BrE-3 antigen in 90% of the tissue samples examined. No tissue sample was BrE-3 antigen negative. Cohorts of a total of 5 patients each received a total of 10 mg, 50 mg, or 100 mg of antibody. At each dose level, 3 patients were studied at NYU and 2 at U.C.Davis. Each dose consisted of 2 mg conjugated antibody labeled with approximately 5 mCi of In-111 and nonconjugated BrE-3 antibody. Infusions of cold antibody over 1 hour followed by radiolabeled antibody over another hour were performed with minimal complications. At NYU, 2 patients experienced mild allergic reactions within 24 hours of infusion. In another patient with extensive liver involvement a Grade 1 liver function test elevation was seen.

Serial imaging was performed to assess localization and obtain pharmacokinetic measurements for dosimetry. Images were performed at 2 hours after the completion of the radiolabeled antibody infusion, approximately 24 hours, 48-72 hours and 8-10 days. On a per lesion (31 lesions) basis the detection rate was 86% in the 9 NYU patients. There was

1 false positive and one lesion remains unconfirmed at this time. 92% of bony lesions, 100% of lung lesions, 33% (1/3) liver lesions, and 100% (2/2) chest wall lesions. Overall 13/15 (86%) of patients showed localization of at least one tumor.

Serum sampling and urine collections were also performed. The fraction of injected dose excreted in the urine by 8 days after infusion averaged 34% ± 19% (range 13-72%). Higher amounts of radioactivity were excreted by patients who had circulating antigen-antibody complexes detected in their serum by HPLC. Biological half life of radiolabeled antibody in the serum averaged 59.4 ±12.4 hours across all patients:

Ab dose level	T1/2 biol (avg) hours	S.D.	range
10 mg	60.9	9.8	50.4-69.9
50 mg	46.3	13.8	35.6-61.8
100 mg	71.0	14.6	62.9-77.3

From the pharmacokinetic measurements and imaging performed in the murine BrE-3 trial, estimates of radiation dosimetry for the In-111 labeled antibody and also extrapolated from the In-111 labeled antibody to the Y-90 labeled antibody.

	In-111 average dose rads/mCi	S.D. rads/mCi	Y-90 average dose rads/mCi	S.D. rads/mCi
brain	0.29	0.28	1.13	1.89
kidneys	0.83	0.74	6.66	6.52
liver	1.30	0.46	9.34	3.56
lungs	0.95	0.46	8.69	5.79
ovaries	0.61	0.11	2.28	0.99
red marrow	0.56	0.45	5.91	6.26
bone surface	1.05	0.71	2.78	1.88
spleen	1.48	0.85	14.84	11.41
urinary bladder	0.54	0.06	2.03	0.43
total body	0.45	0.11	2.14	0.49

Tumor uptake of the ^{111}In-BrE-3 ranged from 0.3 - .002 % ID/g at 24 hours. Dose estimates using the biodistribution of ^{111}In-BrE-3 extrapolated to an ^{90}Y labeled antibody ranged from 1.97 rads/ mCi administered for a large tumor which was not visualized on scan to 590 rads/ mCi .

We have begun a Phase I localization trial of ^{111}In-MX-DTPA humanized BrE-3. To date two patients have been studied completely. Blood and urine pharmacokinetics.have been studied:

	Dose administered	% activity in plasma as antigen-antibody	T1/2 plasma (hrs)	AUC (mCi •hrs)
01HBR	4.12 mCi	15.6%	24.05 ± 4.5	2.20
02HBR	2.99 mCi	2.9%	a=23.3 ± 258.5 b=100.7± 793.8	4.39

03HBR	4.99 mCi	0%	a =3.4 ± 59.3 b =62.4± 641.2	5.39
04HBR	4.85 mCi	0%	a =16.8 ± 34.0 b =192.6± 1825.4	7.18
05HBR	4.75 mCi	0%	a =2.4 ± 13.3 b =103.5± 102.0	6.13

Imaging studies demonstrated an overall sensitivity for known lesions of 85.7% . An additional three lesions have been identified on antibody scan and subsequently confirmed.
Overall Imaging results:

	True Positives	False Negatives	Total lesions	Sensitivity
Lymph nodes	7*	1	8	87.5
Liver	9	2	11	81.8
Lung	9	2	11	81.8
Bone	28*	4	32	87.5
Other	1		1	
Total	54	9	63	85.7

*Each of these categories includes one previously unsuspected, and confirmed, metastatic site.

Using scan biodistribution data and serial blood and urine samples, radiation dose estimates have been made for the ^{111}In-huBrE-3 and extrapolated to ^{90}Y-huBrE-3:

Dose estimates based on biodistribution of In-111 MX-DTPA huBrE-3 (rads/mCi)

**Indium-111
(rads/mCi)**

	01HBR	02HBR	03HBR	04HBR	05HBR	average	S.D.
kidneys	1.99	2.73	1.68	3.22	2.41	2.41	0.61
liver	5.91	2.71	3.03	2.17	2.03	3.17	1.58
lung	1.01	0.81	0.83	1.34	1.33	1.06	0.26
ovaries	0.51	0.46	0.41	0.52	0.56	0.49	0.06
red marrow*	0.5	0.45	0.51	0.66	2.09	0.84	0.70
spleen	3.46	2.08	1.69	1.9	1.74	2.17	0.73
urinary bladder	0.75	0.37	0.34	0.42	0.38	0.45	0.17
whole body	0.621	0.48	0.45	0.52	0.54	0.52	0.07

**Yttrium-90
(rads/mCi)**

	01HBR	02HBR	03HBR	04HBR	05HBR	average	S.D.
kidneys	14.9	27.6	14.6	33.8	23	22.78	8.27
liver	46.9†	19.3†	22.2†	14.7	13.4	23.30	13.66
lung	5.85	5.99	6.39	13.7††	13.2	9.03	4.05
ovaries	1.5	1.45	1.27	1.62	1.26	1.42	0.15
red marrow*	2.02	1.9	3.89	5.69	9.85	4.67	3.29
spleen	37.4	19.5	16.10	16.6	15.2	20.96	9.33

urinary bladder	1.81	1.07	1.09	1.31	0.94	1.24	0.34
whole body	2.82	2.27	2.01	2.35	2.38	2.37	0.29
tumors	101.35	89.63	92.8/81.4	**	43.7	81.78	22.45

*based on blood

†Patients 2 and 3 had liver metastases. Patient 1 manifested diffuse metastases on CT within 3 months.

†† multiple lung metastases

**No measurable tumors visualized on planar images. Dosimetry could not be calculated.

Immunogenicity has been examined qualitatively in serum samples obtained out to 3 months after antibody administration. Serum samples were incubated with radioiodinated or radioindium labeled huBrE-3 and then subjected to HPLC:

Levels of human anti-humanized antibody in patients receiving 50 mg huBrE-3 (ng/ml):

	Baseline	1 week	5 weeks	3 months
01HBR	0	26.4	n.a.	21.8
02HBR	0	0	0	0
03HBR	0	0	3.7	0
04HBR	0	0	0	0
05HBR	0	0	0.3	0

C. Protocol Phase I trial of Y-90 BrE-3 antibody in advanced breast carcinoma:

C.1 Overview

These studies are focused on the development of an effective treatment for breast cancer using radioimmunoconjugates. Because of its very favorable specificity, the results of preclinical studies demonstrating significant therapeutic activity, and the clinical data which suggests good tumor localization and promising dosimetry, we will use antibody BrE-3.

The timeline (see below) outlines our plans for the project period according to the following specific aims:

Specific Aim 1: To determine the toxicity profile and maximum tolerated dose of fractionated radioimmunotherapy using ^{90}Y- labeled huBrE-3 antibody in a Phase I trial in patients with advanced breast carcinoma.

Specific Aim 2: To examine the biodistribution and pharmacokinetics of both ^{111}In labeled BrE-3 and ^{90}Y labeled BrE-3 and the marrow toxicity of the ^{90}Y labeled immunoconjugate.

a) To measure the radiotoxic effect of intravenously administered radioimmunoconjugates directly using hematopoietic progenitor assays.

b) To correlate the marrow and bone localization of ^{111}In with that of ^{90}Y after coadministration of immunoconjugates labeled with each of these radioisotopes.

c)To specifically examine the radiation dose to the marrow estimated for an ^{90}Y immunoconjugate using the biodistribution of ^{111}In BrE-3 in relation to the actual effect on marrow as assessed by hematopoietic progenitor assays and peripheral blood counts.

This study will be a Phase I trial of dose fractionated ^{90}Y-humanized BrE-3. This isotope has been selected because of its pure ß emissions and relatively good tumor retention. A dose escalation schedule for the therapeutic isotope will be followed to evaluate maximum tolerated dose. Because ^{90}Y cannot presently be imaged satisfactorily, we will coinfuse ^{111}In-BrE-3 at a constant dose with the first administration. The designated dose of ^{90}Y will be administered on Day 1. Then using blood levels and whole body counting to determine clearance of ^{90}Y, a second dose (and possibly third dose) will be administered to return to the initial level of radioactivity. Clinical monitoring studies will be carried out to examine the pharmacokinetics and distributions of the ^{90}Y and ^{111}In radioimmunoconjugates. We will assess distribution after the first administration with planar and SPECT imaging, and after each administration determine pharmacologic parameters using serial blood and urine sampling, and tumor biopsies, where possible. We will perform marrow aspirations at the end of the first cycle. We will determine amounts in serum of non-antibody bound radionuclide, degradation products, and intact antibody, for both isotopes, to assess stability and metabolism. Based on data available to date, we anticipate that the behavior of the two conjugates will be similar, with differences (e.g., bone uptake) being predictable. We anticipate that dose-limiting toxicity for this radioconjugate will be myelosuppression.

The studies proposed are based on the use of humanized BrE-3, presently the most promising of the human milk fat globule membrane antigen monoclonal antibodies because of its high expression on breast tumor cells, low levels of available antigen shed into the circulation, preclinical studies demonstrating therapeutic efficacy in model systems, and preliminary Phase 1 localization and pharmacokinetic data which show favorable blood pharmacokinetics and good tumor localization.

Timeline

Years 2-3:   Specific Aim 1 - Phase I trial of 90-Y-BrE-3
                 Specific Aim 2 - Biodistribution and Dosimetry

C.2   Schema

Number of Patients	Dose huBrE-3(mg)		Radioactivity.	
	Unlabeled	Radiolabeled *	^{111}In	^{90}Y
3	38	2 /10	5 mCi	3.0 mCi/ m^2 X 2
3	38	2/10	5 mCi	3.0 mCi/ m^2 X 3
3	38	2/10	5 mCi	4.5 mCi/ m^2 X 2
3	38	2/10	5 mCi	4.5 mCi/ m^2 X 3

* 2 mg of ^{111}In huBrE-3-benzyl methyl DTPA and up to 10 mg of ^{90}Y BrE-3-benzyl methyl DTPA . (The necessary amount of conjugated huBrE-3 will be used and additional nonconjugated huBrE-3 will be added to total 10 mg for the ^{90}Y MX-DTPA huBrE-3)

C.3   Patient eligibility

C.3.1 Patients must have histologically confirmed, metastatic or recurrent breast carcinoma which expresses BrE-3 antigen.

C.3.2 Patients must have measurable or evaluable disease.

C.3.3 Karnofsky performance status of $\geq$ 70 % (ECOG 0,1,2)

C.3.4 Patients must have adequate organ function as defined by:

C.3.4.1 Neutrophil count $\geq$ 2500, platelet count > 100,000, hemoglobin > 9 gm/100ml

C.3.4.2 Bilirubin < 2.0.

C.3.4.3 Creatinine $\leq$ 2.0 or creatinine clearance $\geq$ 40 ml/ minute.

C.3.4.4 Normal chest radiograph or pO2 $\geq$ 80mm Hg on room air and involvement by tumor of $\leq$25% of pulmonary parenchyma as assessed by CT.

C.3.5 No evidence of active infection which requires antibiotic therapy.

C.3.6 Patients entering this study must be 3 weeks post chemotherapy or radiation therapy and have recovered fully from the toxic effects. Patients may not be on concurrent chemotherapy or radiation therapy. Patients must have failed at least 1 prior standard chemotherapy regimen.

C.3.7 Patients with no prior exposure to monoclonal antibodies or serum which is non-reactive to huBrE-3.

C.3.8 Patients must be at least 18 years of age.

C.3.9 Women of child-bearing potential must have a negative pregnancy test.

C.3.10   Evidence of the BrE-3 antigen on immunohistochemistry using BrE-3 antibody in at least 50% of the tumor cells.

C.3.11   Serum BrE-3 antigen level $\leq$ 10µg/ml or a total of 25 mg/total plasma volume

C.3.13   If there is evidence of pulmonary metastases, patients must have adequate pulmonary function (pO2 $\geq$ 80mm Hg on room air) as measured on standard pulmonary function tests and involvement of $\leq$25% of pulmonary parenchyma.

C.4   Exclusion criteria

C.4.1 Patients with evidence of an active second malignancy are not eligible. Patients with a history of a second malignancy, but no evidence of active disease related to this malignancy, may be considered eligible at the discretion of the investigator.

C.4.2 Clinically significant cardiac disease (New York Heart Association Class III/IV)

C.4.3 Serious infection requiring treatment with antibiotics or other serious concurrent illness.

C.4.4 Concurrent steroid therapy.

C.4.5 Pregnancy or lactation

C.4.6 Survival expectancy less than 12 weeks

C.4.7 Active CNS tumor involvement precludes eligibility . This includes spinal cord involvement.

C.4.8 Evidence of extensive skeletal metastases as assessed by bone scintigraphy ;> 25% of the axial skeleton. Bilateral pelvic (sacroiliac) metastases will exclude the patient from eligibility.

C.4.9 Prior exposure to monoclonal antibodies and evidence of anti-huBrE-3 antibody.

C.4.10 Prior bone marrow transplant

C.4.11 Patients with a history of prior irradiation of >25% of their bone marrow will be excluded.

C.5 Drug Information

C.5.1 The monoclonal antibody will be provided by the Cancer Research Fund of Contra Costa, in a final concentration of about 6.9 mg/ml mg/ml. It will be prepared from tissue culture supernatants in hollow fiber bioreactors. It is purified using Protein A and Q Sepharose columns. Purified product will be sterilized through a O.2 u filter. General safety, sterility, pyrogenicity, polynucleotides, mycoplasma, and adventitious virus contamination were tested in accordance with a Notice of Claimed Investigational Exemption for a New Drug (IND) (Office of Biologics, U.S. Food and Drug Administration). All clinical studies will be performed with material prepared under an IND.

C.5.2 The methyl benzyl DTPA conjugated BrE-3 for radiolabeling will be provided by Cancer Research Fund of Contra Costa (CRF) in a concentration of 2 mg/ml. Sterile, low pyrogenic solutions of acetate buffer and sodium/calcium EDTA in saline will be prepared at NYU. The Indium-111(sterile, low pyrogenic) will be purchased INS.IPA from Amersham, Inc. The Yttrium-90 (sterile, low pyrogenic) will be purchased from Pacific Northwest National Laboratory

C.6 Immunoconjugation

Radiolabeling of antibody will be performed under the supervision of Dr. Elissa Kramer in the laboratory located in the Division of Nuclear Medicine of Tisch Hospital.

C.6.1 Chelate

A Methyl benzyl DTPA huBrE-3 conjugate will be provided by CRF. The purity of benzyl DTPA huBrE-3 has been demonstrated by reduced and non-reduced SDS-PAGE stained with coomassie brilliant blue and HPLC sizing column (Indium-111 labeled). The average number of DTPA's per BrE-3 molecule will be approximately 1.1 as estimated using cold competing Indium.

C.6.2 Labeling with ^{111}In

Methyl benzyl DTPA BrE-3 will be radiolabeled under an IND protocol by mixing 2 mg of the antibody chelate with 5-10 mCi of acetate buffered ^{111}In Cl$_3$ for 20 minutes at room temperature. The reaction mixture is then challenged with a 5mM sodium/calcium EDTA solution.

C.6.2 Labeling with ^{90}Y

In addition, Methyl benzyl DTPA BrE-3 will be radiolabeled under an IND protocol by mixing 10 mg of the antibody chelate with the requisite mCi of acetate buffered ^{90}Y chloride for 20 minutes at room temperature.

The labeled preparations (both ^{111}In and ^{90}Y) will be purified by gel filtration on a previously autoclaved 10 cm Sephadex G-80 columns. The fractions corresponding to the immunoglobulin fraction will be pooled and diluted into 0.9% saline solution containing 1% human serum albumin (total volume of 18 ml)

C.6.3 Quality control

A 0.1 ml of the product will be withdrawn for radiochemical purity and immunoreactivity determinations.

Three milliliters of the final products will be withdrawn for USP sterility testing and for pyrogen testing.

The radiochemical purity of the labeled antibody will be determined by ITLC chromatography using O.9% saline/5 mM EDTA eluant. The radiochemical purity of the final product will be >90% for patient administration. Criteria for radiochemical purity and apyrogenicity will be met before the labeled antibody can be administered to the patient. Immunoreactivity of this preparation will be determined using an antigen bead radioimmunogen procedure by applying infinite antigen excess (17). The minimum acceptable immunoreactivity will be 60%. The chromagenic assay test for pyrogens will be within acceptable limits for patient dosing. The sterility test will be performed according to the guidelines prescribed in the United States Pharmacopeia.

C.6.4 Preparation for infusion

Any unlabeled antibody to be administered will be mixed with the radiolabeled
antibody in a total volume of 250 ml of 0.9% saline solution containing 1%
human serum albumin.

C.6.5 Storage

The radiolabeled MoAb BrE-3 will be stored at 2° - 8° C in a shielded
environment while the quality control procedures are in progress. Upon receiving
a satisfactory report about the radiochemical purity and an acceptable level of
pyrogens in the labeled MoAb, it will be made available in the Department of
Nuclear Medicine for administration to the patient.

C.6.6 Drug Accountability

A record of receipt, usage and disposition of huBrE-3 and methyl benzyl DTPA
huBrE-3 will be kept in the Division of Nuclear Medicine. A record of receipt,
usage and disposition of all radionuclides related to this protocol will be kept in
the Division of Nuclear Medicine.

C.7 Study Design

All patients will undergo an evaluation for the purposes of determining and measuring other
sites of evaluable disease. This includes a thorough history and physical examination,
blood counts and chemistry surveys, routine chest x-ray and electrocardiogram, and
urinalysis, computerized tomograms (CT scans) of appropriate areas, bone scan, and, if
there are pulmonary metastases, PFT's . A baseline bone marrow aspiration will be
obtained. In addition, assays for free circulating antigen which is recognized by the
therapeutic antibody will be performed, as well as assays for human antibodies against
huBrE-3 antibody. This will be a study of escalating amounts and serial doses of ^{90}Y
radioactivity labeled to huBrE-3 antibody. Cohorts of 3 patients at each level will be
studied according to the schema detailed below. Pharmacokinetics and serial scanning to
determine the biodistribution and radiation dosimetry will be performed. Toxicity and
response will be assessed using standard criteria (detailed below). If a response is
demonstrated to the first administration in a patient, repeat administration at the same dose
level will be considered.

C.7.1 Informed consent

The protocol will be discussed in detail with each patient prior to enrollment and
written informed consent according to the guidelines of the Institutional Board of
Research Associates of New York University will be obtained.

C.7.2 Antibody administration

Patients accepted on this protocol will receive an infusion consisting of a tracer
dose of ^{111}In labeled antibody together with a therapeutic dose of ^{90}Y labeled
antibody (Day 1). The ^{111}In labeled antibody will be used to establish
biodistribution patterns of this antibody, the degree of tumor localization, and
dosimetric parameters and will be compared with that of the ^{90}Y labeled BrE-3
and other forms of ^{90}Y in blood, urine, and when possible, bone marrow

biopsies and tumor biopsies. The ^{111}In and ^{90}Y infusion will be repeated after 1 week (Day 8) and with further dose escalation, at Day 15 as well.

For this Phase I study all patients will be out-patients at either Tisch Hospital or Bellevue Hospital Center unless anticipated external radiation exposure rates exceed the radiation safety guidelines. They will be monitored in the Division of Nuclear Medicine in the hospital up to 2 hours post infusion. All patients will receive coinfusions of 5 mCi of ^{111}In BrE-3-methyl benzyl DTPA and escalating doses of ^{90}Y BrE-3-methyl benzyl DTPA as detailed in the schema followed by serial gamma camera images following both infusions. Blood clearance and imaging for organ uptake studies will also be performed on each patient. Based on our recent experience in the Phase I trial of In-111 -murine and humanized BrE-3 monoclonal antibodies, we have determined that a 50 mg dose of antibody may be administered without undue toxicity and that this will permit delivery of radiolabeled antibody to tumor as long as circulating antigen levels do not exceed 10 µg/ml.

C.7.2.1 Dose escalation

This will be a dose escalation study to determine the MTD of ^{90}Y methyl benzyl DTPA BrE-3 antibody with cohorts of 3 patients entered initially at each of the following dose levels:

Radioactivity	
^{111}In	^{90}Y
5 mCi	3.0 mCi/ m^2 weekly X 2
5 mCi	3.0 mCi/ m^2 weekly X 3
5 mCi	4.5 mCi/ m^2 weekly X 2
5 mCi	4.5 mCi/ m^2 weekly X 3
5 mCi	6.0 mCi/ m^2 weekly X 2
5 mCi	6.0 mCi/ m^2 weekly X 3

C.7.2.2 Criteria for dose escalation (Figure 1)

Dosages will not be escalated over successive treatment courses for individual patients. All of the initial three patients at a given dose level will be observed for four weeks before additional patients are added. All patients at each dose level will be observed for a period of four weeks after the last radioimmunoconjugate administration before any patients are enrolled at the next higher dose level.

It is estimated that approximately 18-24 patients per year will be enrolled using this schema.

C.7.2.2.1 If no grade III(non-hematologic) or grade IV toxicity (by common toxicity criteria, see Appendix 1) is observed among the first three patients at a given dose level, the dose will be increased for the successive group of three patients.

C.7.2.2.2 If at least one patient among the first three patients at a given dose level experiences Grade III(non-hematologic) or IV toxicity (hematologic and < 1 week in duration), an additional three patients will be treated at that level.

C.7.2.2.3 If one patient experiences Grade IV (nonhematologic) toxicity or if one patient experiences Grade IV hematologic toxicity of ≥ 1 week duration, no more patients will be treated at that dose level and the maximum tolerated dose will have been exceeded. Three more patients will be treated at the next lower dose level, if necessary, to reach a total of 6 patients at that lower dose level.

C.7.2.2.4 Of the 6 patients at a given dose level, if only one patient experiences dose limiting toxicity (Grade III nonhematologic or Grade IV hematologic lasting < 1 week), the dose will be increased for the successive group of three patients.

C.7.2.2.5 Of the 6 patients at a given dose level, if 2 patients experience Grade IV(hematologic and < 1 week in duration) toxicity, or if three patients experience Grade III nonhematologic toxicity , or if 2 patients experience Grade III nonhematologic and 1 experiences Grade IV hematologic toxicity (<1 week in duration), no more patients will be treated at that dose level and the maximum tolerated dose will have been exceeded.

C.7.2.2.6 The dose level at which two patients experience Grade III (nonhematologic) toxicity or one patient experiences Grade III (nonhematologic) toxicity and one Grade IV(hematologic and lasting <1 week toxicity), will be classified as the maximum tolerated dose.

C.7.2.2.7 If we reach a situation where the MTD seems to be placed between an extremely safe level and an unacceptably toxic level, we will enroll a cohort of patients at an intermediate dose level according to this schema.

C.7.2.2.8. At the MTD, a total of 6 patients will be treated in order to provide adequate evaluation of radioisotope content of marrow and marrow response at that level.

C.7.2.3 In-111/ Y-90 BrE-3 methyl benzyl DTPA administration procedure

For the administration of the initial dose levels of ^{111}In and ^{90}Y hu BrE-3 methyl benzyl DTPA patients may be outpatients. Patients will be hospitalized in the CRC at Bellevue Hospital if and when the level of administered radioactivity (^{90}Y and ^{111}In) exceeds permissible limits for outpatients. All administrations will be performed under clinical observation. Hypersensitivity skin testing will not be performed prior to each antibody infusion in each patient. Data from the

National Cancer Institute suggests no correlation between local erythema and induration at the site of skin tests and subsequent systemic allergic reactions to mouse antibody infusions (K.Foon, personal communication). Skin testing may also increase the likelihood of sensitization to mouse antigens.

C.7.2.3.1 Infusion

Intravenous tubing will be pretreated with 0.9% NaCl and 1% human serum albumin. After placement of an intravenous line, the radiolabeled/nonradiolabeled huBrE-3, at the dose described above,will be infused over the course of one hour depending on rate- dependent side effects in a volume of 250 ml of 1% human serum albumin in normal saline.Vital signs will be taken every 15 minutes during the infusion, and every one hour post infusion until stable. A thorough cardiopulmonary physical examination will be done prior to and at the conclusion of antibody infusion. Medications including acetaminophen, diphenhydramine, epinephrine, and corticosteroids will be kept at hand for treatment of allergic reactions should they occur. An emergency cart will be at hand.

C.7.2.3.2 Radiation safety precautions will be observed by all personnel :

Gloves will be worn when handling samples of blood, urine, or other body fluids.

Laboratory specimens will be labeled with radioisotope labels.

There are no restrictions to visitors, including hospital personnel related to isotope administration while the total administered dose of radioactivity and/ or external exposure rate remains within radiation safety guidelines.

The radiation safety precautions and health safety precautions established by institutional radiation safety guidelines will be observed.

For outpatient administration, no special precautions are necessary in the patient's home.

Patients will be treated as in-patients when the level of administered radioactivity or the anticipated external exposure rate exceeds the permissible limit for out-patient administration. The patients will be monitored by radiation safety on a daily basis. The patient will be confined to hospital until radioactivity levels as determined by radiation safety permit their discharge.

C.7.3 Duration of treatment

C.7.3.1 Patients without progression and less than dose limiting toxicity after one course of treatment, should be retreated at the same dose of Y-90 BrE-3 methyl benzyl DTPA 4 weeks weeks after the last dose. In-111 labeled BrE-3 methyl benzyl DTPA will be coinfused with the first dose of each repeat cycle of administrations.

C.7.3.2 The following criteria must be met before retreatment:

1. Serum negative for human anti-human BrE-3 antibody
2. Neutrophil count $\geq$ 2500
3. platelets > 100,000
4. pO2 $\geq$ 80 mm Hg on room air

C.7.3.3 If persistent hematologic toxicity at 6 weeks prevents retreatment, patients may be treated at 8 weeks if hematologic recovery has occurred.

C.7.3.4 If Grade III (nonhematologic) or Grade IV (hematologic, <1 wk duration) toxicity occurs in a patient with partial or complete response, the patient may be retreated with half the radioactive dose of Y-90 BrE-3 methyl benzyl DTPA received in the initial treatment dose, at the discretion of the investigator. The mass amount of antibody administered at each dose level will be held constant, however.

C.7.3.5 Patients whose sera test positive for human anti-huBrE-3 antibody may be retreated at the discretion of the investigator.

C.7.3.6 Patients who do not show progression 6 weeks after the second course of therapy and have less than Grade III toxicity may be retreated at the discretion of the investigator. There will be no more than 3 cycles of radiolabeled antibody administered to any one patient.

C.7.4 Off study criteria

C.7.4.1 Progressive disease at 6 weeks on study. (Patients will be followed for a minimum of 8 weeks for toxicity or until toxicity resolves.) If patients have progressive disease prior to 6 weeks, patients may be removed from the study earlier at the discretion of the investigator.

C.7.4.2 Intercurrent illness which prevents further administration of ^{90}Y BrE-3 methyl benzyl DTPA.

C.7.4.3 Unacceptable toxicity

C.7.4.4 Decision of patient to withdraw from the study

C.7.4.5 General or specific changes in the patient's condition which render the patient unacceptable for further treatment in the judgement of the investigator.

C.8 Management of toxicity and reporting of Adverse Drug Reactions (ADR's)

C.8.1 Hazards and Protection

C.8.1.1 Radiation

C.8.1.1.1 Hazards

The long term toxicities of intravenous radiolabeled monoclonal antibody therapy are not known. Any of the chronic toxicities associated with

external beam whole abdominal irradiation such as bowel or bladder fibrosis, liver dysfunction or peritonitis with adhesions could occur potentially. Similarly, chronic toxicities such as pneumonitis and fibrosis from lung irradiation might occur at these dose rates.

The most likely subacute toxicities which might be expected on the basis of other radiolabeled antibody therapy trials (1,17, 26) are hematologic and include pancytopenia and fever. Other toxicities which might be occur depending on the localization of the antibody include pulmonary toxicity, diarrhea and peritonitis. Hematologic toxicities are the major toxicity expected and will most likely determine the maximum tolerated dose for Y-90 huBrE-3 methyl benzyl DTPA. These will be graded according to NCI guidelines. (Please see Appendix 1)

The amount of radiation exposure with In-111 BrE-3 methyl benzyl DTPA is minimal and is not expected to result in any toxicity. However it is anticipated that the radiation exposure from Y-90 will cause the dose limiting toxicity and most likely will be hematologic.

C.8.1.1.2 Monitoring

CBC's with differential will be monitored weekly for 8 weeks or until counts normalize. Hepatic and renal toxicity will be monitored by blood chemistries. Pulmonary toxicity will be monitored by pulmonary function tests and arterial pO_2. Serial physical examinations will be performed to monitor for other organ system toxicity.

C.8.1.2 BrE-3 antibody

C.8.1.2.1 Toxicity associated with the antibody

In the two patients who have received In-111 huBrE-3 to date, no allergic or toxic reactions have been observed by clinical or laboratory criteria. Of the 15 patients who received 5 mCi of In-111 labeled murine BrE-3 (5 each at 10 mg, 50 mg, and 100 mg) at NYU Medical center or U.C. Davis, 2 have shown mild allergic reactions which were treated with oral antihistamines and resolve. An additional patient has shown a slight increase in liver function tests. One showed a slight drop in hemoglobin most likely due to blood drawing.

Of the seven patients who have received ^{90}Y BrE-3 at doses of 6.25 mCi/m2 (n=3), 9.25 mCi/m2 (n=3), and 12.25 mCi/m2 (n=1), one episode of flushing during administration, and one patient with a single hive within 24 hours of administration were observed. Hematologic toxicity was observed in one patient at 4 weeks after antibody administration and 3 days after chemotherapy (Grade 1) and a transient Grade 4 thrombocytopenia was observed in the heavily pre-treated patient at the highest dose level. Pretreatment assays of CFU's in her bone marrow showed very little hematopoietic reserve.

In the first six evaluable patients who have received In-111 humanized BrE-3 (5 mCi/50 mg), no antibody-related toxicities have been observed. One patient developed a Grade III thrombocytopenia 8 days later which was

more likely related to the concurrent radiation therapy to a large portion of the spine and extensive bony metastases replacing functional marrow.

HAHA responses have been seen in three patients (see above). In two this was a trace amount at five weeks post infusion only. In one HAHA was measured as early as one week.

Both Mc5 and KC4G3 are similar murine monoclonal antibodies which bind to an epitope very similar to the one recognized by BrE-3. These have been used more extensively in patients.There has been no unexpected toxicity associated with Mc5 in diagnostic imaging trials. As with other murine monoclonal antibody trials in humans the overall toxicity with Mc5 was mild and explainable in terms of allergic reactions or occasional low grade fever. The allergic reactions included low grade fever, chills, pruritus, occasional rash, and occasional headache. There was no evidence of hepatic or renal toxicity. No change in pancreatic enzymes or thyroid function tests was seen.

No unexpected toxicity has been seen with KC4G3 in either diagnostic imaging or therapeutic trials. In nonsmall cell lung cancer patients, a dose escalation study was performed with unlabeled antibody. 10-500 mg was administered twice weekly for 4 weeks. 3 of 15 patients experienced reactions, but with no long term sequelae. Furthermore, these reactions occurred after repeated administration and total doses of 230, 780 or 2000 mg. This included transient hypotension in a patient who developed high HAMA (IgE and IgG) titers. Acute dyspnea occurred in one patient (780 mg). A serum sickness like syndrome occurred in the patient who received 2 g. of antibody. We will be administering significantly lower doses of antibody protein in this protocol.

Repeated administration of murine MoAbs more than 10-12 days apart can result in allergic reactions including serum sickness and anaphylaxis.

HAMA may result from the administration of murine monoclonal antibodies such as BrE-3. We have seen HAMA responses in 4/6 patients evaluated to date. HAHA in response to humanized BrE-3 has been seen in trace amounts at 5 weeks and 3 months after infusion. This will be monitored as detailed under Serial Observations.

C.8.1.2.2   Precautions

The radiolabeled antibody will be subject to quality control procedures including immunoreactivity testing, pyrogen testing (limulus amoebocyte lysate tests), sterility, and ITLC.

The antibody will be infused over a period of 1 hours with trained medical personnel in attendance. Vital signs will be monitored during the infusion and for an hour after. A baseline cardiopulmonary physical examination will be performed prior to and after administration of the radiolabeled antibody. An intravenous line which is placed for administration of the radiolabeled antibody will be kept in place. Antihistamines, epinephrine, and corticosteroids will be kept at hand. There is an emergency cart available in the Division of Nuclear Medicine. Patients with grade 1 or 2 toxicity may continue on study at the discretion of the investigator. In the

event of more serious reactions, the antibody infusion will be stopped and treatment with subcutaneous epinephrine, intravenous steroids, respiratory assistance other resuscitative measures will be instituted. No further antibody will be administered.

C.8.2 Reporting of adverse drug reactions

Adverse drug reactions to In-111 or Y-90 BrE-3 methyl benzyl DTPA or the unconjugated, unlabeled BrE-3 antibody will be reported by phone to the Principal Investigator, and IDB (301-496-7957) within 24 hours :

a) All serious toxicity (Grade 3 and 4) which may be due to antibody administration

b) All fatal events

c) First occurrence of any toxicity regardless of grade other than Grade I fever .

d) All grade 4 reactions and patient deaths while on treatment

Written reports to follow within 10 working days to :
Investigational Drug Branch
P.O. Box 30012
Bethesda, Maryland 20824

Previously known Grade 2 and Grade 3 reactions are to be reported to the NCI in writing using the "NCI adverse reactions form for Investigational Agents" within 10 working days.

All adverse reactions will also be reported to the Institutional Review Board.

C.9 Criteria for Response

C.9.1 Methods of malignant disease evaluation

C.9.1.1 Measurable Bidimensional:

Malignant disease measurable in two dimensions by rulers or calipers (metric system) with surface area determined by multiplying the longest diameter by the greatest perpendicular diameter(i.e., metastatic pulmonary nodules, hepatic metastases, lymph nodes, and subcutaneous masses). Malignant disease with sharply defined borders visualized by ultrasonography, computerized axial tomography or magnetic resonance imaging is considered measurable. Repeat studies will be performed at the same pretherapy (baseline) site(s).

C.9.1.2 Measurable, unidimensional.

Malignant disease measurable in one dimension by rulers or calipers (metric system) (e.g., mediastinal adenopathy, malignant hepatomegaly, or abdominal masses)

Mediastinal and hilar involvement may be measured if a preinvolvement chest radiograph by subtracting the normal mediastinal or hilar width on the preinvolvement radiograph from the onstudy width containing malignant disease (Alternatively, this may be handled as two-dimensional disease if chest CT is available)

Malignant hepatomegaly may be measured if the liver descends >5 cm below the costal margin by adding the measurements below the costal margins and the tip of the xyphoid.

C.9.1.3   Nonmeasurable, evaluable

Malignant disease evident on clinical (physical or radiographic) examination, but not measurable by ruler or calipers (e.g. lymphangitic or confluent multinodular lung metastases, skin metastases, ascites or pleural effusions known to be caused by peritoneal or pleural metastases and uninfluenced by diuretics, bone scans, gallium scans, deviated or obstructed ureters, or gastrointestinal tract and poorly defined masses by ultrasonography, CT, or MRI).

Photographs should be taken prior to and during therapy to document response of externally visible disease.

Malignant ascites known to be caused by malignant involvement of the peritoneum and uninfluenced by diuretics may be followed by serial abdominal girths measured through a specified fixed point

Serial x-rays of lymphangitic or confluent multinodular lung metastases, pleural effusions or bone metastases should be compared to evaluate response.

Bone and other scintigraphic scans can be used to evaluate response.

Chemical parameters and biologic markers will be measured but will not be used to evaluate response. Normalization of hyperbilirubinemia known to be caused by malignant disease may be used as an evaluable response.

C.9.2 Objective Response Criteria

C.9.2.1   Complete response:

Complete disappearance of all clinically detectable malignant disease for at least four weeks. Bony metastases, radiographically detected prior to therapy, must show normalization or complete sclerosis of lytic metastases with a normal bone scan. If only bone scan is positive, this must show normalization.

C.9.2.2   Partial response:

Greater than or equal to 50% decrease in tumor area for at least 4 weeks without appearance of new areas of malignant disease.

C.9.2.2.1   Measurable, bidimensional

$\geq$ 50% decrease in tumor area or a 50% decrease in the sum of the products of the perpendicular diameters of multiple lesions in the same organ site for at least 4 weeks.

C.9.2.2.2   Measurable, unidimensional

$\geq$ 30% decrease in linear tumor measurement for at least 4 weeks.

A partial response of malignant hepatomegaly has occurred if the sum of the liver measurements below the costal margins, in the midclavicular lines, and the tip of the xyphoid decreases by $\geq$30%.

C.9.2.2.3   Nonmeasurable, evaluable

Definite improvement in evaluable malignant disease estimated to be in excess of 50% and agreed upon by two independent investigators

Serial evaluations of chest x-rays and physical measurements should be documented in the records and by photograph when practical

The response should last for at least 4 weeks.

A partial response of bony metastases occurs if there is a partial decrease in the size of the lesions or decreased density of blastic lesions, lasting for at least 4 weeks.

C.9.2.3   Stable

No significant change in measurable or evaluable disease for a least 4 weeks ($\geq$ 12 weeks for bony metastases) including:

No significant increase in size of any known malignant disease

No appearance of new areas of malignant disease

This includes decrease in size of bidimensional disease of < 50% or decrease in size of unidimensional disease of < 30% or increase in malignant disease of < 25% in any site.

No deterioration of ECOG performance status $\geq$ 1 level related to the malignancy

C.9.2.4   Progressive disease

Significant increase in size of lesions present at the start of therapy or after a response or appearance of new metastatic lesions known not to be present at the start of therapy or stable objective disease associated with a deterioration in ECOG performance status of $\geq$ 1 level related to malignancy.

C.9.2.4.1   Measurable, bidimensional or unidimensional disease

≥25% increase in the sum of the products of the 2 dimensions of the individual lesions in an organ

≥50% increase in the size of the product of the 2 diameters if only one lesion is available for measurement and was ≤ 2 cm^2 in size at the start of therapy.

≥25% increase in the sum of the liver measurements below the costal margins and xyphoid.

Appearance of new malignant lesions.

C.9.2.4.2   Nonmeasurable , evaluable

Definite increase in the sum of the areas of malignant lesions estimated to be > 25%

Appearance of new malignant lesions (e.g. bony metastases)

C.10   Study parameters and serial observations (see Appendix 2):

C.10.1 Initial Evaluation

C.10.1.1 Immunohistochemistry will be performed on biopsy or surgical material from primary tumors or metastases to determine the presence and extent of the expression of BrE-3 antigen. Tumor cells must stain positive for the antigen.

C.10.1.2 Patients will undergo complete medical history and physical examination including height, weight, and performance status.

C.10.1.3 Complete CBC including differential blood count and platelet count, PT, PTT.

C.10.1.4 Electrolytes, total protein, albumin, calcium, phosphorus, glucose, creatinine, uric acid, BUN, alkaline phosphatase, total bilirubin, LDH, SGPT and SGOT.

C.10.1.5 Urinalysis

C.10.1.6 Chest x-ray and other x-rays or scans as clinically indicated to document state of disease. A radionuclide bone scan will be performed to assess extent of bony disease. A chest CT will be performed to assess the extent of lung disease.

C.10.1.7 EKG

C.10.1.8 A serum sample (10-15 cc) will be collected pre-injection, aliquoted, and frozen at -20° C for determination of HAHA. (Other samples will be collected at other times points for storage as shown in the serial observations).

C.10.1.9 Bone marrow aspiration will be performed to provide baseline assessment of bone marrow precursor cells and to assess by PCR for the presence of tumor.

C.10.1.10 If the chest radiograph or chest CT are abnormal, pulmonary function tests including arterial pO_2 will be performed to assess baseline pulmonary function.

C.10.1.11 A serum sample will be collected pre-injection, aliquoted, frozen at -20° C and shipped by overnight mail on ice for determination of BrE-3 antigen levels. This will be used for the initial screening.

C.10.2 Serial Observations and Laboratory Monitoring Schedule

At the time of the infusion of the In-111/ Y-90 labeled BrE-3 the following serial samples and measurements will be performed for pharmacokinetics and to make dosimetric estimates:

C.10.2.1 Blood samples will be obtained for analysis of radioactivity and for RIA analysis of BrE-3 clearance 5, 120 minutes, 4, 24, 48, and 72 hours, 4, 5, 6 and 7 days after the first injection. Thereafter, samples will be obtained 5 minutes, 24 hours, 72 hours and 7 days after subsequent administrations.

C.10.2.2 Urine samples will be collected from O-2, 2-4, 4-24, 24-48, 48-72, 72-96, 96-144, 144-192 hours after the first infusion. For subsequent administrations, urine will be collected over the first 24 hours.

C.10.2.3 Complete CBC including differential blood count and platelet count, PT, PTT, electrolytes, total protein, albumin, calcium, phosphorus, glucose, creatinine, uric acid, BUN, alkaline phosphatase, total bilirubin, LDH, SGPT and SGOT, and urinalysis will be performed on Day 6, Day 13, Day 20 or 21, and week 4, 5, 6, 7 and 8 weeks after the first administration of the Y-90 labeled antibody or until toxicity resolves.

C.10.2.4 Physical examination will be performed on Day 7, 14, 21, and 4, 6 and 8 weeks after the first administration of the Y-90 labeled antibody to assess response in evaluable disease.

C.10.2.5 An additional serum sample for HAHA will be obtained at Day 6, Day 13 (if a third administration is planned), Day 24 and 5 weeks after Y-90 BrE-3 administration. If three doses are to be given, serum for HAHA will also be obtained at Day 20.

C.10.2.6 The anterior and posterior regional gamma camera images which include the chest, abdomen, and pelvis will be obtained at 2, 24, 48 and/or 72 hours and 8, and, optionally, at 10 days after administration of the Indium-111 radioimmunoconjugate. A large field of view gamma camera fitted with a medium energy collimator will be used. Anterior and posterior whole body scans will also be performed archived for later analysis. The data will be processed and stored with a dedicated computer which is available in the Nuclear Medicine

Department to measure regional uptake of radiolabeled BrE-3 in major organs, tumor, and blood pool at the times designated above using region of interest analysis. In addition, SPECT imaging will be performed at 72 hours and/or 7-10 days post administration.

C.10.2.7 An additional bone marrow aspiration and a core biopsy will be obtained at Day 8 and again, when possible an aspiration at 7 days after the last infusion to assess the possibility of further damage. The % injected dose/gram will be measured using both ^{111}In and ^{90}Y. Progenitor assays(CFU-G, CFU-M, CFU-GM,BFU-E, CFU-GEMM) will be performed and compared to baseline studies. Marrow samples will also be assessed by PCR for tumor cells. The marrow in the biopsy on day 8 will be assessed for ^{90}Y and ^{111}In content (%ID/g). The bony portion of the core biopsy will be processed to isolate bone for evaluation of ^{90}Y and ^{111}In content (%ID/g). If there is evidence of later hematologic toxicity, an optional fourth bone marrow aspiration will be obtained.

C.10.2.8 When accessible tumor is present, biopsy of tumor will be obtained 6-8 days after administration of the ^{90}Y methyl benzyl DTPA BrE-3. This tumor biopsy will be analyzed for % injected dose/g of tumor, immunohistochemical evidence for antibody and antigen. These biopsies will be performed by Dr. Richard Shapiro, Dr. Daniel Roses, or Dr. Matthew Harris

C.10.2.9 Pulmonary function tests including arterial pO_2 will be performed prior to any repeat cycle of radioimmunotherapy.

C.11   Methods

C.11.1   Image Analysis/Pharmacokinetics/Dosimetry Estimates

C.11.1.1 Pharmacokinetic Analysis

The pharmacokinetic analysis of labeled and unlabeled monoclonal antibody movement into the tumor and through each patient will provide a manageable and useful summary of the data that are collected. The pharmacokinetic analyses will:

1) Provide information concerning the biodistribution of both In-111 labeled methyl benzyl DTPA huBrE-3 and Y-90 labeled methyl benzyl DTPA huBrE-3 for radiation dosimetric estimates based on the MIRD formalism.

2) Provide information concerning the pharmacokinetics of Y-90 methyl benzyl DTPA huBrE-3 and products of its metabolism.

3) Provide a basis for correlating the pharmacokinetics of the In-111 labeled methyl benzyl DTPA BrE-3 and the Y-90 labeled methyl benzyl DTPA BrE-3 to determine the potential for using In-111 labeled chelate conjugated antibody to predict the biodistribution and dosimetry of Y-90 labeled methyl benzyl DTPA BrE-3.

In general, the efficiency of elimination of the monoclonal antibody via the urine, and in toto from the blood, will be described using renal antibody clearance and total antibody clearance from the blood, respectively. Uptake of the radiolabeled antibody by the organ(s) of interest will be described over time. The clearances and uptake will provide information which allows an approximation of the biodistribution of the antibody and the radioisotopes. This will most likely relate to the formation of antigen-antibody complexes in the blood as well as the stability of the two different labels on the antibody . Specifically, the estimates based on this information will be correlated with direct measurements of uptake in marrow and, when possible, tumor. These estimates will also be related to the effect on marrow function.

4) Provide a basis for comparing the blood pharmacokinetics of Y-90 MX-DTPA huBrE-3 over repeat administrations.

C.11.1.1.1   Biodistribution

In order to study the distribution of conjugated monoclonal antibody labelled with Indium-111, whole body scintigraphy will be obtained at 2, 24, 48, and/or 72 hours and one week after each injection in the Nuclear Medicine Department. Regions of interest will be established and counts per pixel calculated for those organs which have significant uptake and for a background region in order to calculate relative uptake ratios. Previous studies have demonstrated significant accumulations of radioactivity in liver, spleen, kidneys, and lungs. In addition, serial blood sampling will provide an estimate of whole body distribution of radiolabel.

Attenuation of activity will be corrected for by transmission images obtained just prior to antibody administration for each patient using a flat field source filled with a known concentration of ^{111}In. Alignment of digital transmission and emission images will be performed using fiduciary markers and a two-dimensional image registration algorithm within *qsh*, an image handling toolkit which runs under a UNIX operating system (53). Whole body counts will be obtained from anterior and posterior whole body scans. Activity within an organ will be determined using the attenuation corrected geometric mean of conjugate views (60). The consistency of detection sensitivity of the camera will be checked on a daily basis at the time of the patient imaging by imaging a standard at a fixed distance to yield a system calibration factor (14). This standard will be used to convert region of interest data into absolute amounts of radioactivity and also to express activity within regions of interest as percent injected dose. From this data, the fraction of the radiolabel resident in each organ can be closely estimated.

For estimation of tumor radiation absorbed dose, the uptake will be calculated from the attenuation-corrected geometric mean if the localization is observed on both anterior and posterior views . If the tumor localization is observed on only one view, the activity will be calculated based on the effective point source assumption(66) .

The standard MIRD formulation will be used to calculate the radiation absorbed dose based on the calculated activity localized either in normal organs or tumor.

C.11.1.1.2   Evaluation of Urine/Plasma

In general, the goal of these studies is to provide better understanding of the biodistribution of Y-90 labeled methyl benzyl DTPA BrE-3 and to better understand the similarities and differences between the In-111 and Y-90 when infused labeled to this chelate conjugated antibody so that eventually the In-111 labeled compound may be used to predict the behavior of the Y-90 labeled compound. For each first infusion of radiolabeled monoclonal antibody, blood samples will be drawn at 5 minutes ,2, 4, 6, 24, 48, and 72 hours, 4, 6, and 8 days post infusion. Urine samples will be collected from O-2, 2-4, 4-24, 24-48, 48-72, 72-96,96-144, 144-192 hours postinfusion. For subsequent infusions, urine will be collected over the first 24 hours and blood samples will be obtained at 5 min. after the end of infusion, 24 hours, 72 hours and 6 days.

Y-90 and In-111 labeled antibody will be measured in serial serum samples and in urine to provide estimates of whole body distribution of the radiolabel. Time activity curves will be generated and half-times calculated.

Total radioactivity (dpm/ml) will be measured in each serum sample and urine sample. We will count aliquots of blood and urine obtained at multiple time points in comparison to a standard made from the administered dose. This is then expressed as % injected radioactivity cleared or excreted over time, allowing for normal radioactive decay by standard decay formulas. In addition, HPLC will be performed on plasma samples to determine the form in which each radioisotope exists, specifically free isotope, chelated, antibody bound, or transchelated (especially In-111). This data will be used in the pharmacokinetic analysis.

C.11.1.1.3   Blood pharmacokinetics

The distribution of monoclonal antibody will be followed by radioactivity determinations from the timed blood samplings. These will be converted into monoclonal antibody concentration based on the specific activity determination of the labeled BrE-3 antibody . Nonlinear pharmacokinetic models which run on a PC , PCNONLIN (Statistical Consultants, Lexington Ky) will be used for the analysis of the data. Previous studies with "first generation" monoclonal antibody imaging reagents have shown that postinfusion blood concentrations can follow either patterns that resemble one or two compartment models that reflect both the dose and the physiologic characteristic of the patient. Indeed, analysis of In-111 BrE-3 has shown a monoexponential clearance from serum. Initial parameter estimates will be obtained using the JANA curve stripping programs (Statistical Consultants, Lexington Ky). These estimates will be applied to the appropriate model to best fit the time/concentration MAB data with respect to the duration of the infusion as well as the IV route of

administration. The nonlinear models in PCNONLIN use LaGrangian techniques to reduce the total variance of appropriate parameters and yield the key pharmacokinetic parameters. These include half-lives for each compartment (e.g. alpha, and beta for 2 compartment models), the area under the plasma concentration versus time curve (AUC), the apparent volume of distribution, intercepts (e.g. A & B in a 2 compartment model). The time of distribution , total body clearance, and renal clearance are derived using urine excretion data. The clearance values will reflect the volume of blood and or urine from which the monoclonal antibody is removed per unit time (ml/min).

C.11.1.1.4   Dosimetry calculations

Organ data for the In-111 labeled methyl benzyl DTPA huBrE-3 will be obtained from the scans using region of interest analysis, attenuation correction of data, and the geometric mean of the anterior and posterior views of each organ.

The blood and organ radioactivity data will be examined using a computer program "S" designed to fit the data to a curve or curves. This program provides estimates of slopes and intercepts for each exponential component. The slopes and intercepts for the exponentials will be used with the standard MIRD dosimetry formulations (as implemented by MIRDOSE2, Oak Ridge) to make dosimetric estimates.

Excretion rate plots (urinary excretion rate of radioactivity vs. time) will be examined in a manner similar to the analysis of the blood radioactivity data.

Finally, when possible, we will use the data obtained from imaging studies from the tumor site to estimate tumor dosimetry. Count density from the scans will be used to estimate change in tumor uptake over time. More helpful will be data obtained if and when accessible tumor is biopsied. This can be compared with scan data to develop dosimetric estimates.

C.11.1.2 Analysis of Marrow

Bone marrow aspiration will be performed prior to infusion and at Day 8. Marrows will be counted for both Y-90 and In-111. Aspirates will be evaluated with marrow progenitor assays (BFU-E, CFU-G, CFU-M, CFU-GM, CFU-GEMM). Aspirates will also be submitted for PCR to evaluate for tumor cells.Bone marrow biopsies will be evaluated for marrow content of radioactivity. A portion of the bone marrow biopsy will be purged of bone marrow and then counted for radioactivity.

Radioactivity concentrations in marrow will be examined in relation to activity measured from scans over vertebral regions of interest and to measured blood activity.

C.11.1.3 Analysis of Biopsies

In patients with accessible tumors, when feasible, we will biopsy tumor 6-8 days after infusion of radiolabeled antibody to evaluate DPM.'s/gram

tissue weight and immunohistochemical analysis of tumor antigen expression and uptake by antibody.

C.11.1.4 Image evaluation

The categorical evaluation of the radionuclide scans will be scaled based on the ratings of two observers. This will be performed for each patient and for each known lesion.

C.11.2 Determination of circulating BrE3 antigen

Circulating BrE-3 epitope in the serum of treated patients will be determined by a competitive serum assay with the BrE-3 epitope on the solid phase. Microtiter plates precoated with a source of BrE-3 epitope will be presented with stoichiometric quantities of BrE-3 and the serum added in adequate dilution. After an overnight incubation BrE-3 bound to the solid phase will be detected by a radioiodinated anti-mouse Ig antibody. Results obtained will be compared to a standard curve originated against increasing concentrations of the epitope and expressed as μg/ml of protein equivalent breast epithelial mucin.

C.11.3 HPLC Analysis of Urine and Plasma

The analysis by gel permeation chromatography of plasma and urine samples will allow the assessment of the degree of labeled antibody and free label as well as possible immune complexes at the various time points followed during the pharmacokinetic sampling. Samples will be applied to a 7.8 mm by 600 mm BioSil SEC-250 column equilibrated with 50 mM phosphate buffer and 0.1M Na_2SO_4 pH 6.8 with a flow rate of 0.6 ml/min. Detection will be at 280 nm and 0.6 ml fractions will be collected for determination of radioactivity. BioRad molecular weight callibration standards (ribonuclease, ovalbumin, myoglobin, gamma globulin, and thyroglobulin) will be used.

Analysis by HPLC will determine levels (specific activity) of serum and/or urine ^{111}In Iabeled BrE-3 and ^{90}Y Iabeled BrE-3.

C.11.4 Immunohistochemical analysis of biopsied tumor samples and previously obtained tissue for screening.

Immunohistochemical staining is performed under Dr. Jonathan Melamed's supervision with a full time registered histology technician performing all antibody staining. Tumor tissue will also be subject to routine histopathologic staining. Histopathology of previously obtained tumor specimens for eligibility screening and examination of any tumor biopsies will include:

Gross examination of freshly obtained tissue:
1. Site.
2. Size

Microscopic evaluation will include:
1. Histologic subtype.
2. Histologic grade.

3. Nuclear grade.

Immunohistochemical evaluation will include:
1. % of cell positive (0 to 100%)
2. Intensity (0 to 3+)

Control will be evaluated for:
1. % of cell positive (0 to 100%)
2. Intensity (0 to 3+)

C.11.5 Histopathological examination of bone marrow

Bone marrow aspirates will be assessed histologically for cellularity and for the presence of metastatic carcinoma. Cellularity will be expressed as the percentage of the cellular marrow elements in relation to the total of the cellular elements and the bone marrow fat. Cellularity normally decreases with age. The results will also be reported semiquantitatively as normocellular, slightly hypercellular, markedly hypercellular, slightly hypocellular, markedly hypocellular, or acellular.

C.11.6 Immunoreactivity

The degree of immunoreactivity will be assessed after each radiolabeling procedure. The assay employs a single radiolabeled antibody concentration that is coupled with the use of antigen-coated beads to produce a determination the amount of non-specific binding along with the percent of total binding. The assay utilizes negative controls which consist of bovine serum albumin coated beads . A brief description of the protocol for immunoreactivity testing follows below:

Reagents: 1% BSA RIA grade (Sigma) in 10 mM potassium phosphate buffered 0.9% sodium chloride containing 0.1% sodium azide, pH 7.2 (1% BSA-PBS), wash buffer consisting of 0.15M Sodium chloride, 10 mM phosphate, 0.,1% azide, 0.05% Tween 20. Beads (6.4 mm polystyrene, Precision Ball Co) coated respectively with albumin for non-specific binding (NSB) and antigen for specific binding (Bmax).

Protocol: Radiolabeled antibody is diluted to yield the equivalent of 25,000 cpm per ml in assay buffer. Tubes are prepared to contain in triplicate the NSB, the Bmax and the total counts (TC). To each tube with the exception of the total counts, 0.2 ml of the diluted radiolabeled antibody is added. After incubation overnight at room temperature, the solution is aspirated from each of the NSB and Bmax tubes followed by the addition of 3 ml of the wash buffer. The wash buffer is aspirated off and the tubes including the TC are counted in a gamma counter. The labeling is considered acceptable at values of 50% binding or better with less than 3% non-specific binding and a CV for triplicate analyses of each sample.

C.11.7   HAHA

C.11.7.1 IgG

Anti-human IgG and antiidiotype are determined by mixing radiolabeled huBrE-3, or matched isotype human IgG with the patients serum . After incubation, samples are processed on an HPLC column which gives sufficient resolution to identify Ab-Ab complexes..

C.11.8 Hematopoietic progenitor assays

These assays will be performed on all marrow aspirations obtained.

Samples will be collected into preservative free heparin (100 u/ml), mixed well, and gently expelled into disposable, sterile, screw-topped vials. Samples are held on ice until delivery to the core laboratory.

Marrow samples are diluted 4 to 5-fold with RPMI 1640 + 10% heat-inactivated calf serum. 8 ml of the diluted sample are then layered over 4 ml of Ficoll-Hypaque for density centrifugation. Cells at the interface are removed, diluted gently with medium, centrifuged and washed. Cells are suspended in IMDM + 10% heat-inactivated fetal calf serum and counted. Cytospin preparations are made of the cell inocula for subsequent examination if warranted. Cells are suspended to a concentration of 1×10^5 cells/ml for marrow. Aliquots of 0.4 ml of cell are then added to 4 ml of freshly thawed plating medium. These mixtures are vortexed and plated into bacteriological dishes (1ml/ dish; 3 dishes per sample). The cultures are incubated in a well-humidified chamber at 37°C for 12-14 days and read for colony formation. Colonies are distinguished on the basis of established criteria. Cytospin preparations are made of representative colonies from each sample series to verify identifications.

C.12   Statistical Considerations

D.12.1 <u>Duration of Study :</u> We expect to enroll 18 patients in this study; however, the maximum sample size is 24 patients. It is expected that 3 patients will be accrued every 7 weeks. We may be able to repeat therapy in a few patients. Although the duration of the study will depend on the number of doses investigated and whether additional patients are required at one or more levels, the first Phase I study should be terminated within 12-13 months.

D.12.2 <u>Data analysis :</u> As demonstrated in Appendix 3 several outcomes will be measured in this study. As this is a phase I study, the results of this study are not meant to be definitive but, rather to gain information for future plans. For this reason, formal statistical inference testing will not be performed on these data; however, a number of descriptive analyses will be presented.

The major clinical endpoints of this study will be toxicity. The MTD is that dose at which one third of patients experience dose limiting toxicity (DLT). Escalations are planned in groups of three patients, with an additional three patients to be added at the first indication of the MTD.Three patients will be studied at the first dose level. All three patients must be studied for at least 6 weeks. If none of these three patients experience DLT, the treatment dose will be escalated to the next higher level in the three subsequent patients. If one of three patients experience DLT at a given dose, three more patients will be added at the same dose. If none of the three additional patients experience DLT, the dose will be escalated for the next patient treated. If two patients of the total six patients at a given dose level experience DLT, then this dose will be classified as the MTD. Once three patients experience Grade III (nonhematologic) or two patients experience Grade III (

nonhematologic) and one has Grade IV(hematologic, <1 week's duration) at a given dose level, then the MTD will have been exceeded. Similarly, if one instance of Grade IV (hematologic > 1 week's duration or nonhematologic) occurs, then the MTD will have been exceeded. A total of 6 patients will be studied at the preceding dose level.

Although clinical response is not a major endpoint of the study, this study may generate preliminary information about therapeutic efficacy. Levels and duration response will be tabulated for each patient.

The serial serum and urine measurements are taken at 5 minutes post infusion, 2 hours, 4 hours, 24 hours, 48 hours, 72 hours , at days, 5, 6, and 8. For each patient the measurements from each source will be plotted over time in order to assess 1) how the relation between ^{111}In and ^{90}Y varies over time for each source of both measurements; and 2) whether the functions of time are consistent over the patients. Graphical displays will also be used to determine whether the half-time clearance of radioactivity has a consistent relation between ^{90}Y and ^{111}In over patients for serum and urine measurements. For subsequent infusions only ^{90}Y will be measured at the end of infusion, 24 hours, 72 hrs and Day 6. These measurements will be plotted over time in order to assess 1) how the ^{90}Y varies from the first infusion to the second or third and 2) whether functions of time are consistent over the patients.

Pharmacokinetic parameters for ^{111}In- labeled antibody and for ^{90}Y-labeled antibody will be calculated and will include: peak plasma level (C max), time to peak plasma level (t max), plasma elimination half-life (t 1/2), area under the plasma concentration-time curve (AUC). These parameters for ^{90}Y- and ^{111}In- labeled antibody will be tabulated for each patient.

The gamma camera imaging is performed for ^{111}In at 2, 24, 48, and/or 72 hours, at day 8, and optionally, at day 10. The images will be analyzed using region-of-interest analysis for organ uptake of ^{111}In radioactivity. For each patient these measurements from each organ will be plotted over time in order to assess whether the functions of time are consistent over the patients.for organ distribution.

Radioactivity levels for both ^{111}In and ^{90}Y will be derived at day 8 from serum, urine, marrow aspirate, marrow biopsy and for ^{111}In by gamma camera imaging. These measurements will be graphically presented in order to 1) determine whether an obvious relation exists over all patients between the ^{111}In value and the corresponding ^{90}Y value from the same source; 2) to explore possible relations among the five ^{111}In measurements over all patients; 3) to ascertain whether the same relations exist among the subset of four ^{90}Y measurements. These graphical displays, along with the relevant set of Kendall's correlation coefficients, will give an indication of whether the ^{111}In labeled compound can be used to predict the activity of the ^{90}Y labeled compound, specifically at day 8 and whether the blood (serum) levels can be used to predict bone marrow levels of radioactivity specifically at day 8. Correlation coefficients will be calculated using data from all patients, regardless of the dose level.

Baseline values of and percent changes in CFU's will also be tabulated for all five precursors for each patient. The Wilcoxon signed-rank test will be performed for each CFU assay in order to assess whether significant changes have occurred indicating toxicity. However depending on sample sizes, meaningful changes may not be supported by

statistical significance. Changes in CFU's will be correlated with levels of accumulated ^{90}Y levels in the marrow and bone and with radiation dose estimates by means of graphical displays and Kendall's correlation coefficients.

Radionuclide scan findings will be tabulated for each patient based on the ratings of two observers. This will be done on a per patient basis and for known lesions, on a per lesion basis.

The analysis for safety will include examination of all changes in physical examination and laboratory evaluation. All clinical and laboratory parameters will be monitored for safety according to NCI toxicity guidelines (Appendix 1). All adverse experiences reported during study therapy will be tabulated.

C.13   Data Management

Weekly meetings of the co-principal investigators will be held to assess data, logistics, and coordinate the group's effort. Monthly meetings of all investigators and support staff will be held to review progress and potential problems. Dr. Ceriani will be consulted on a monthly basis to report on progress of the NYU group and coordinate with the direction and progress of the overall program. Data will be transferred to Dr. Ceriani on a biweekly basis via modem.

D.   Potential risks

D.1   Monoclonal antibody

Human administration of BrE-3 has been very limited to date. We have administered between 10 and 100 mg of BrE-3 (2mg of the methyl benzyl DTPA chelate conjugate form with 8 mg non-conjugated) labeled with 5 mCi of ^{111}In to a total of 15 patients to date. Two patients experienced mild, transient allergic reactions. One patient with a large amount of metastatic liver disease experienced a transient Grade 1 elevation of liver function tests. In one patient a 1 gm drop in hemoglobin was observed, most likely due to blood drawing.

Both Mc5 and KC4G3 are similar murine monoclonal antibodies which bind to an epitope very similar to the one recognized by BrE-3.

There has been no unexpected toxicity associated with Mc5 in diagnostic imaging trials. As with other murine monoclonal antibody trials in humans the overall toxicity with Mc5 was mild and explainable in terms of allergic reactions or occasional low grade fever. The allergic reactions included low grade fever, chills, pruritus, occasional rash, and occasional headache. There was no evidence of hepatic or renal toxicity. No change in pancreatic enzymes or thyroid function tests was seen.

No unexpected toxicity has been seen with KC4G3 in either diagnostic imaging or therapeutic trials. In nonsmall cell lung cancer patients, a dose escalation study was performed with unlabeled antibody. 10-500 mg was administered twice weekly for 4 weeks. 3 of 15 patients experienced reactions, but with no long term sequelae. Furthermore, these reactions occurred after repeated administration and total doses of 230, 780 or 2000 mg. This included transient hypotension in a patient who developed high

HAMA (IgE and IgG) titers. Acute dyspnea occurred in one patient (780 mg). A serum sickness like syndrome occurred in the patient who received 2 g. of antibody.

We will be administering significantly lower doses of antibody protein in this protocol.

The radiolabeled antibody will be subject to quality control procedures including immunoreactivity testing, pyrogen testing (limulus amoebocyte lysate tests), and ITLC.

The antibody will be infused over a period of 2 hours with trained medical personnel in attendance. Vital signs will be monitored during the infusion and for an hour after. A baseline cardiopulmonary physical examination will be performed prior to and after administration of the radiolabeled antibody. An intravenous line which is placed for administration of the radiolabeled antibody will be kept in place. Antihistamines, epinephrine, and corticosteroids will be kept at hand. There is an emergency cart available in the Division of Nuclear Medicine. Patients with grade 1 or 2 acute toxicity may continue on study at the discretion of the investigator. In the event of more serious reactions, the antibody infusion will be stopped and treatment with subcutaneous epinephrine, intravenous steroids, respiratory assistance other resuscitative measures will be instituted. No further antibody will be administered.

D.2 Radiation

Biodistribution of ^{111}In labeled methyl-benzyl DTPA-BrE-3 monoclonal antibody has been studied in 15 patients with metastatic or recurrent breast carcinoma. Biodistribution arr organ pharmacokinetics have been used to develop radiation dosimetry estimates for ^{111}In labeled methyl-benzyl DTPA-BrE-3. These have also been extrapolated to 90Y-MX-DTPA BrE-3. The average whole body dose from 111 In MX DTPA is estimated to be 0.45 rads/mCi administered and from ^{90}Y-MXDTPA BrE-3 the estimated whole body dose is 2.14 rads/mCi administered. The marrow doses for ^{90}Y-MXDTPA BrE-3 ranged from 0.44 rads/mCi to 14 rads/mCi administered.

D.2.1 Radiation Safety/Precautions

Gloves will be worn when handling samples of blood, urine, or other body fluids.

Laboratory specimens will be labeled with radioisotope labels.

There are no restrictions to visitors, including hospital personnel related to isotope administration while the total administered dose of radioactivity and/ or external exposure rate remains within radiation safety guidelines.

The radiation safety precautions and health safety precautions established by institutional radiation safety guidelines will be observed.

For outpatient administration, no special precautions are necessary in the patient's home.

Patients will be treated as in-patients when the level of administered radioactivity or the anticipated external exposure rate exceeds the permissible limit for out-patient administration. The patients will be monitored by radiation safety on a daily basis.

The patient will be confined to hospital until radioactivity levels as determined by radiation safety permit their discharge.

A history of contraceptive use or a negative pregnancy test will be required of all women of child-bearing potential entered in this protocol.

D.3   Venopuncture:

A total of 100 ml of whole blood will be drawn over a period of a week for pharmacokinetics. The early time point will be drawn through a small peripheral intravenous line to minimize the number of needle sticks. In addition, approximately 25 ml will be required for routine CBC and chemistry as part of the initial evaluation and again for each weekly follow-up for 6- 8 weeks.

Venopuncture and blood drawing will be performed by experienced medical personnel. All laboratory specimens will be labeled with radioisotope labels. Efforts will be made to minimize the number of punctures necessary to obtain the requisite blood samples.

D.4   Confidentiality:

The records of the subjects entered into this study will be kept in a locked file. Beyond the study personnel, only representatives of the FDA or Coulter Immunology will have access to these files.

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AD_____

GRANT NUMBER: DAMD17-94-J-4176

TITLE: Radioimmunotherapy of Metastatic Breast Cancer Using
Radiolabeled Tumor Specific Antibodies

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Introduction

The following is a progress report for Grant No. DAMD17-94-J-4176, entitled "Radioimmunotherapy of Metastatic Breast Cancer Using Radiolabeled Tumor Specific Antibodies" for the period of September 1, 1994 to August 1, 1994. I have previously notified you of some changes in the original proposal which are detailed below. The changes are directed toward improving the potential efficacy of radioimmunotherapy in breast cancer. The changes, however, have resulted in delay in the initiation of the clinical trials because we are awaiting the availability of the humanized version of the radioimmunoconjugate.

The overall goal of the project is to develop effective therapy for breast cancer using radioimmunoconjugates. In our initial proposal we planned to conduct a phase I study of murine 90 Yttrium MX-DTPA BrE-3 in women with metastatic breast cancer. Based on previous preliminary results, doses of ^{90}Y MX-DTPA of 12.25 mCi/m^2 in one patient resulted in significant hematologic toxicity, the development of HAMA and no antitumor effect. Over the last year we have been exploring methods to deliver radioimmunotherapy more effectively. One potential method is to be able to deliver repeated doses of radioimmunotherapy. A humanized version of the antibody would significantly reduce the immunogenicity of the monoclonal antibody and potentially allow for dose fractionation. Recently, humanization of both the constant and variable portion of the BrE-3 antibody has been accomplished with preservation of the CDR's of the antibody in their murine form, maintaining the affinity of the antibody. The humanized version of BrE-3 (huBrE-3) has been designed at the Cancer Research Fund of Contra Costa by Drs. Joseph Couto and Roberto Ceriani in collaboration with E. Padlan (NIH). The purpose of the design was to diminish the immunogenicity of the frameworks while strictly maintaining the antigen binding affinity. This required that all amino acid interactions that might affect the conformation of the antigen binding surfaces be left intact. In compliance with this goal, all the framework amino acids have been mutated from murine to human identities except for those amino acids judged to be important for antigen binding. All of these "murine" residues which have been preserved have either inwardly pointing side chains or make contacts with the opposite antibody chain. Therefore, their side chains should not be available for binding in internalization by B-cells and, therefore, should not be immunogenic. Eight of these residues make CDR contacts; the consequences of replacing these "murine" amino acids which are important for antigen binding cannot be predicted.

In spite of these remaining "murine" residues, the VL and VH frameworks of the humanized antibody are, respectively, 93% and 90% identical to the corresponding human frameworks (kII and HIIIc). This similarity to the human frameworks exceeds that of many human antibodies for their own consensus. The affinity of huBrE-3 for the BrE-3 antigen exceeds that of the murine antibody by three-fold. HuBrE-3 antibody stains human breast tumors in histological paraffin-embedded sections, clearly discriminating between normal and transformed cells.

Another potential strategy aimed at increasing the potential efficacy of radioimmunotherapy is by combining this with other agents such as chemotherapy. At NYU, a model drug that we have helped develop is the topoisomerase 1 inhibitor topotecan (topo). Topoisomerase-1(topo 1) is a unique target for cancer chemotherapy. It is a nuclear enzyme involved in unwinding of supercoiled DNA and is integrally involved in a host of cell functions including replication and transcription. Drug interaction with this enzyme converts topo-1 into a "cellular poison" and results in progressive cell death. Many effective chemotherapy drugs (e.g. doxorubicin and the other anthracyclines, podophyllotoxins, anthracene drugs) act on topoisomerase-2, an enzyme involved in double strand DNA unwinding and breakage. Furthermore, many of these same drugs are cross-resistant and are susceptible to resistance mediated by the Multi-Drug Resistance phenotype. Topo-1, however, which inhibits the resealing step of single strands breaks of DNA, is inhibited by camptothecin (CPT), a natural product from the plant *Camptotheca acuminata,* and its analogs. This compound has been part of the Chinese pharmacopeia as a plant extract for many years. The plant's active component, CPT converted into a sodium salt, was tested in the clinic as an anticancer drug in the early 1970's but abandoned because of unpredictable toxicity. Now it is recognized that the salt exists as carboxylated inactive form of CPT. More recently, interest in CPT has been stimulated by the awareness that its mechanism of action involves interaction with topoisomerase-I through formation of a "cleavable complex" involving a DNA:topo-1:drug configuration. With the development of newer analogs that are potentially more effective and less toxic than the parent drug, the opportunity exists to investigate this novel class of compounds in human cancer therapy and its mechanism of action.

We have conducted a Phase I study utilizing a novel schedule for administration of topo, under sponsorship of CTEP. In this study topo was given as an ambulatory infusion in low doses, continuously for up to a 21-day duration. We proposed this study based on our observations that prolonged subcutaneous administration of 9-AC suspension to mice bearing human tumor xenografts, resulted in unprecedented anti-tumor activity, with little or no toxicity (1). To date we have treated 43 patients in a phase I study of dose escalating topo, reaching a 21 day duration at 0.7 mg/m2/day. We have determined the MTD for heavily pretreated patients to be 0.53 mg/m2/day for 21 days, increasing dose intensity by >50% compared to conventional (daily x 5) administration schedule. We have also observed unprecedented activity in a phase I study, including partial remissions in patients with ovarian and breast cancer (previously received 4-5 regimens including taxol) and renal cancer (2). This schedule deserves further investigation in the clinic and we now have plans to combine this schedule with radioimmunotherapy in phase I studies.

While studies of topo-1 inhibitors in combination with radioimmunotherapy have not yet been reported, experimental models with external beam radiation therapy show that the combination of these two modalities enhance cell kill in cell culture and *in vivo* (3-7) It has been postulated that the synergism between the topo-1 inhibitors and ionizing radiation is due to the ability of topo-1 inhibitors to interfere with repair of radiation-induced DNA damage (8). Ionizing radiation sensitizes cells to topo-1

inhibitors by slowing their progression through S-phase, thus, increasing the number of cells in S-phase (5). The most optimal effects *in vivo* have been seen when the topo-1 inhibitor is given shortly before the irradiation (6), or concurrently with continuous application (9,10). Findings of synergism between topo and ionizing radiation in experimental models of lung cancer (4) have led to an ongoing clinical trial of combined external beam radiation therapy and topo in patients with mediastinal cancer, primarily lung cancer.

While external beam irradiation of loco-regional disease is possible in a disease like primary non-small cell lung cancer, this is a less feasible approach with respect to metastatic breast cancer which may be more widely disseminated. Radioimmunoconjugates provide a vehicle for targeting therapeutic doses of radiation to widely dispersed tumor throughout the body. Similarly, the above principles of synergy will apply to radiation delivered by this method as well as by external beam, but with improved therapeutic index. The potential for increased toxicity of the two modalities is also present. Although this has not occurred consistently in experimental models of radioimmunotherapy and radiosensitizers (9), it has been observed with 5-bromodeoxyuridine(11)and with hypoxic cytotoxins (12). Clinically, increased toxicity has been seen in the more radiosensitive organs within the radiation port when radiosensitizers are used (13), For instance, increased pulmonary toxicity has been observed in patients undergoing lung irradiation with radiosensitizer administration(10). It should be noted, however, that topo-1 inhibition is not equivalent mechanistically to such radiosensitizers and these data are of unknown importance to the studies proposed here.

Body

Over the last year, huBrE-3 has been produced in large quantity by the Cancer Research Fund of Contra Costa. Purification of one gram of huBrE-3 has been completed. Necessary safety testing has been successfully performed. This includes testing for sterility and mycoplasma contamination. The antibody has also been conjugated by Dr. Quadri at M.D, Anderson. The chelate is identical to that utilized with the murine monoclonal antibody, MX-DTPA, produced by IDEC pharmaceuticals. Successful conjugation has been completed and an IND was submitted to the FDA several months ago for which we recently gained approval.

In preparation for clinical trials using huBrE-3 at NYU, we have obtained New York University approval to conduct a phase I study using ^{111}In-MX-DTPA huBrE-3 in patients with advanced breast cancer. The objectives of this study are:

1) To assess the toxicity and efficacy of using a humanized ^{111}Indium labeled monoclonal antibody to localize tumor in patients with advanced breast cancer. To utilize nuclear medicine scanning to assess the ability of the monoclonal antibody to image sites of known disease in patients with advanced breast cancer.

2) To study the pharmacokinetics of this radiolabeled monoclonal antibody to develop dosimetry estimates to assess its potential as a radioimmunotherapeutic agent.

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3) To assess the development of anti antibody response to administration of this antibody and to determine the nature of this response

4) To assess expression of the BrE-3 antigen in human breast tumors by retrospective study of the pathology specimens.

The protocol is submitted as appendix 1. It is similar to our initial radiolocalization trial with the murine antibody. We will study up to 15 patients with advanced breast cancer. Patients entered on the protocol will receive 5 mCi of Indium 111-MX-DTPA huBrE-3(2 mg) plus 48 mg of nonconjugated BrE-3 intravenously over one hour. Images and pharmacokinetics will be performed over the course of one week. In addition, conventional clinical evaluation for toxicity will be performed at baseline, during infusion and at 72 hours after infusion consisting of history, physical examination, and routine blood tests. Anti-antibody response will be assessed at baseline, day 8, 5 weeks, and 3-6 months post antibody administration.

It is likely that we will be able to begin to treat patients by 9/95. We are currently in the process of screening 16 potentially eligible patients for BrE-3 Ag by retrieving tissue from there original surgical specimen and measuring serum levels of BrE-3. We anticipate that we will be able to complete this protocol within twelve months. Following this study, we plan to initiate a radioimmunotherapy trial with ^{90}Yttrium-huBrE-3 alone, or in combination with potentially synergistic agents such as topo or with IL-1 as described in the original grant proposal.

We have done preliminary work to determine the distribution and toxicity of BrE-3 ^{90}Y conjugate. Human breast tumor xenografts were grown in Balb/c nude female mice. Mice were treated with between 150 µCi- 355 µCi ^{90}Y MX-DTPA BrE-3. From these preliminary studies we were able to define the maximum tolerated dose of ^{90}Y MX-DTPA BrE-3 as 266 µCi ^{90}Y in this murine system and expect that 200 µCi will be the starting dose for the combination studies in animals. The potential enhancement of therapeutic index of radioimmunotherapy with a breast-tumor directed radioimmunoconjugate by the addition of a topo-1 inhibitor such as topo or 9-aminocamptothecin will be examined in an animal model of human breast cancer. This xenograft model will help determine the optimal schedules of administration of each modality, and permit preliminary estimates of effects on toxicity. It is expected that this experimental work will provide the basis for a clinical Phase I trial in patients with advanced breast cancer which will capitalize on the advantages of the more effective topo-1 inhibitor chosen, the targeting capability of a radioimmunoconjugate, specifically ^{90}Y- MX-DTPA huBrE-3, and the anticipated synergism between the two therapies.

Preliminary to the combined modality studies, over the last year we have completed several experiments in the mouse model in an attempt to evaluate the efficacy of continuous infusion topotecan. In the fall of 1994, we performed the first experiments on N/PLOR male mice six to eight weeks old. We used 6 mice per

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experimental group. On day 0, each mouse was injected with 3×10^5 of syngeneic mammary tumor MGT cells subcutaneously. On day 1, two groups of 6 mice each had water/topo pumps (Alzet pumps) implanted under the skin, through a small incision. Each pump was programmed for infusion of up to seven days. After the placement of the pump, the skin was stapled and the wound was disinfected with betadine. One group of mice received saline alone and one group received topo at 2.0 mg/m^2 continuous infusion over seven days. One group of mice served as controls and did not receive pumps. At each timepoint for up to 20 days, the topo group had lower tumor volumes than both the saline pump group and the control mice. This is shown in figure 1 in the appendix. Over the next several months these experiments were repeated for verification. A later experiment performed in January, 1995 failed most likely because the Avertin was not fresh and was subsequently reprepared. In addition, the topo dose was higher at 2.4 mg/m^2.

We then performed experiments to evaluate the effect of a more prolonged infusion topotecan on tumorigenesis. On day 0, all mice received 3×10^5 MGT cells injected subcutaneously. On day 1, two groups of six mice each had implantation of the Alzet pump as described above. One group received a saline infusion and the other group received a topo dose of 2.0 mg/m^2. Another group of six mice served as controls (did not have pump implantation). On day 10, a second Alzet pump was inserted in the first two groups of mice with, one group receiving saline and the topo group continuing to receive topo at 2.0 mg/m2 over seven additional days. Because the pumps were not available on day 8, the topo group had a two day period of no drug. The results of this experiment is shown in figure 2 in the appendix. At each timepoint tumor growth in the topo group was inhibited.

The same experiment was repeated in the Spring of 1995, with the exception that male mice were used, and the second pumps were inserted on day 8. In this experiment, the topo group of mice had a continuous infusion of topotecan over 14 days. The results of this experiment is shown in figure 3 in the appendix. In this experiment there was overlap of the tumor volume in the control group without pumps and the topo group. The reason for this was not clear.

The next set of experiments have incorporated IL-1 to assess whether IL-1 has any synergistic effects on tumor growth inhibition with topo and to assess toxicity. Female mice, 6 to 8 weeks old were used in five groups of six randomly selected mice each. On day 0 each mouse was injected with 3×10^5 MGT cells s.c.. On day 1, two groups of mice received IL-1, 0.25 ml intraperitoneal (0.5 ug/mouse). IL-1 alpha rhu has been supplied from Immunex. It is packaged as 100 ug suspended in 0.2 ml saline-BSA. Each vial contains 100 ug-5×10^8 IL-1, 40 mg mannitol, 10 mg sucrose and 1.2 mg tris. Four hours after the IL-1 injection, Alzet pumps were inserted. Two other groups of mice also had Alzet pumps inserted on day 1. As. above the topo dose used was 2.0 mg/m^2. Group 1 received saline pump alone. Group 2 received IL-1 followed by the saline pump. Group 3 received IL-1 followed by topo pump infusion. Group 4 received topo pump infusion alone. Group 5 served as control and did not have pump insertion

or IL-1 injection. On day 8, new pumps were implanted in the topo animals to receive another 7 day infusion of 2.0 mg/m^2. As shown in figure 4 in the appendix, there was no obvious difference in tumorigenesis among the various groups.

Subsequently, we have performed an experiment using IL-1 in a continuous infusion. Other experiments carried out by our colleagues had demonstrated that continuous infusion of IL-1 yielded a higher therapeutic index, lower toxicity and greater hematologic reconstitution than bolus injection. Male mice, 8 to 10 weeks were used. On day 0, five groups of six mice each were injected with 3×10^5 MBT cells, s.c. On day one, four groups of mice had pumps implanted as described above. Each pump was prepared as follows:

	EMPTY	TOPO	IL-1	TOPO + IL-1
Topo	-------	35 ul	-----	35 ul
Saline-BSA	65 ul	65 ul	15 ul	15 ul
IL-1	--------	------	50 ul	50 ul
Water	35 ul	-------	35 ul	-------

The effect of the infusions on tumorigenesis is shown in figure 5 in the appendix. Although not dramatic, the topo plus IL-1 group had the most tumor growth inhibition. White blood cell counts were performed on the IL-1 and IL-1 plus topo group at baseline and on day 5. The WBC count increased by approximately 2-3 fold.

In the coming funding period, we intend to begin combination studies with topo and ^{90}Y MX-DTPA BrE-3 in the nude mouse xenograft model, (human breast cancer cell lines ZR-75 or MX-1). The initial combination studies will examine the possible toxicities that could be additive. We will start at a dose slightly below the MTD(200 uCi BrE-3) defined in our preliminary experiments with this xenograft model. Dosing levels of topo will be selected on the basis of measurement of adequate topo levels by the Alzet pump in the current experiments. Mice will be followed for up to 4 weeks with the BrE-3 dosing regimen set at an initial dose one week after the topo administration. If substantial toxicities result, we will test a dose fractionation schema once a week with half or one third the dose of BrE-3. Based on the acceptable toxicity profile of combination topo and radioimmunoconjugate, the timing sequence of these modalities will be examined with regard to efficacy on tumor growth inhibition. Each of the experiments will incorporate 4 arms: control mice with no drug administration, topo alone, ^{90}Y-MX-DTPA BrE-3 alone and ^{90}Y-MX-DTPA BrE-3 plus topo. Again, animals will be followed for up to 4 weeks. Steady state drug levels of topo and tumor drug levels will be followed. In addition, tumor distribution of BrE-3 will be assessed. Tumor growth will be measured in all the groups and efficacy will be determined by reduction in tumor size. If the IL-1 decreases toxicity and/or improves efficacy, it will

subsequently be incorporated into the preclinical studies. Ultimately, we plan to use the data generated in these combination studies to develop a clinical trial.

Conclusion

In summary, over the last year we have made progress in obtaining a humanized BrE-3 monoclonal antibody chelated to ^{111}Indium which is ready to begin phase I study testing. We plan to initiate a radiotherapeutic trial with ^{90}Y huBrE-3 as soon as we obtain preliminary data regarding safety and feasibility on the ^{111}Indium study. In the original grant application, we planned to initiate a radiotherapeutic study with the murine antibody because we did not expect to have the humanized antibody available. For the reasons outlined above, the humanized antibody has greater potential for therapeutic efficacy and we look forward to begin these studies within the next month.

We plan to initiate combination studies with targeted radioimmunotherapy and chemotherapy, specifically the topo I inhibiter, topotecan. Preliminary work in the mouse model performed this year demonstrated that prolonged infusion topotecan can be administered safely to mice and has antitumor activity. The addition of IL-1 had variable results described above. Over the upcoming year we plan to do more extensive studies in nude mice bearing human tumor xenografts with combination radioimmunotherapy and chemotherapy. We will continue to explore the potential role of IL-1 as a radioprotector. Ultimately, we hope to conduct a clinical trial using combination topotecan and ^{90}Y-huBrE-3.

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AD_____

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Introduction

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Another potential strategy aimed at increasing the potential efficacy of radioimmunotherapy is by combining this with other agents such as chemotherapy. As noted in my previous progress report, at NYU, a model drug that we have helped develop is the topoisomerase-1 inhibitor topotecan (topo). Topoisomerase-1(topo-1) is a unique target for cancer chemotherapy. It is a nuclear enzyme involved in unwinding of supercoiled DNA and is integrally involved in a host of cell functions including replication and transcription. Drug interaction with this enzyme converts topo-1 into a "cellular poison" and results in progressive cell death.

We have conducted a Phase I study utilizing a novel schedule for administration of topo, under sponsorship of CTEP. In this study topo was given as an ambulatory infusion in low doses, continuously for up to a 21-day duration. We have determined the MTD for heavily pretreated patients to be 0.53 mg/m2/day for 21 days, increasing dose intensity by >50% compared to conventional (daily x 5) administration schedule. We have also observed unprecedented activity in a phase I study, including partial remissions in patients with ovarian and breast cancer (previously received 4-5 regimens including taxol) and renal cancer (1). We are currently performing a phase I study evaluating 3 hour paclitaxel and 14 day continuous-infusion topotecan to evaluate the toxicity and response in patients with advanced cancer.

While studies of topo-1 inhibitors in combination with radioimmunotherapy have not yet been reported, experimental models with external beam radiation therapy show that the combination of these two modalities enhance cell kill in cell culture and *in vivo* (2-6). It has been postulated that the synergism between the topo-1 inhibitors and

ionizing radiation is due to the ability of topo-1 inhibitors to interfere with repair of radiation-induced DNA damage (7). Ionizing radiation sensitizes cells to topo-1 inhibitors by slowing their progression through S-phase, thus, increasing the number of cells in S-phase (4). The most optimal effects *in vivo* have been seen when the topo-1 inhibitor is given shortly before the irradiation (5), or concurrently with continuous application (8, 9). Findings of synergism between topo and ionizing radiation in experimental models of lung cancer (3) have led to an ongoing clinical trial of combined external beam radiation therapy and topo in patients with mediastinal cancer, primarily lung cancer.

While external beam irradiation of loco-regional disease is possible in a disease like primary non-small cell lung cancer, this is a less feasible approach with respect to metastatic breast cancer which may be more widely disseminated. Radioimmunoconjugates provide a vehicle for targeting therapeutic doses of radiation to widely dispersed tumor throughout the body. Similarly, the above principles of synergy will apply to radiation delivered by this method as well as by external beam, but with improved therapeutic index. The potential for increased toxicity of the two modalities is also present. Although this has not occurred consistently in experimental models of radioimmunotherapy and radiosensitizers (8), it has been observed with 5-bromodeoxyuridine (10) and with hypoxic cytotoxins (11). Clinically, increased toxicity has been seen in the more radiosensitive organs within the radiation port when radiosensitizers are used (12). For instance, increased pulmonary toxicity has been observed in patients undergoing lung irradiation with radiosensitizer administration (9). It should be noted, however, that topo-1 inhibition is not equivalent mechanistically to such radiosensitizers and these data are of unknown importance to the studies proposed here.

As previously reported, we have conducted experiments in the mouse model and demonstrated the feasibility of administering topotecan as a continous infusion. We also demonstrated an antitumor effect. As described below we have initiated experiments using continuous infusion topotecan and radioimmunoconjugate. The data generated thus far support the use of combination therapy for future clinical trials.

Body

Over the last year, we have initiated a phase I study using ^{111}In-MX-DTPA huBrE-3 in patients with advanced breast cancer (previously submitted to you.). The objectives of this study are:

1) To assess the toxicity and efficacy of using a humanized [111]Indium labeled monoclonal antibody to localize tumor in patients with advanced breast cancer. To utilize nuclear medicine scanning to assess the ability of the monoclonal antibody to image sites of known disease in patients with advanced breast cancer.

2) To study the pharmacokinetics of this radiolabeled monoclonal antibody to develop dosimetry estimates to assess its potential as a radioimmunotherapeutic agent.

3) To assess the development of anti antibody response to administration of this antibody and to determine the nature of this response.

4) To assess expression of the BrE-3 antigen in human breast tumors by retrospective study of the pathology specimens.

Validation of sterility, apyrogenicity, and preservation of immunoreactivity were performed in September and early October 1995. To date, we have submitted the names of 70 patients for immunohistochemistry screening. Thirty-six of those patients have had tissue available and 25 patients have been positive (≥25% cell staining) and are eligible. We studied two patients in November and December 1995 respectively. They each received 2 mg of MX-DTPA huBrE-3 labeled with about 5mCi of Indium 111 plus 48 mg of nonconjugated BrE-3 intravenously over one hour. The antibody infusions were well tolerated. No allergic or toxic side effects were observed. Patients underwent serial whole body counting, gamma camera imaging, plasma and urine sampling over one week in order to assess pharmacokinetics, radiation dose and tumor localization. Imaging was quite promising in both patients. We imaged 22/26 (84.6%) known bone, liver, and lung lesions and identified two sites previously unsuspected (lymph node, bone). Blood and urine pharmacokinetics were also measured (Table 1, Appendix). Blood half-lives have been faster than the average blood half-lives observed in our patients studied with the murine antibody. In our first patient in whom antigen-antibody complex averaged 15.6% of the circulating radioactivity after antibody administration, a single compartment model gave a good fit to the data. A shorter plasma half life and smaller AUC was associated with higher circulating antigen. In our second patient, in whom the antigen-antibody complex averaged only 2.9% of the circulating radioactivity, a two-compartment model gave the best fit for the data. Radiation dose estimates (using standard MIRD formalism) have been made for normal organ and tumor (Table 2, Appendix). Liver uptake is somewhat higher in the patients receiving the humanized BrE-3 than average hepatic uptake of the murine antibody. Immunogenicity has now been studied out to 3 months in our patients. Qualitative analysis of serum incubated with either radioiodinated huBrE-3 or Indium-111 labeled hu BrE-3 demonstrates that compared to baseline serum, there are trace amounts of antibody-antibody formation at 5 weeks and 3 months after antibody infusion. Since these anti-humanized antibodies react equally with the murine BrE-3, we believe that "HAHA" represents an idiotypic response.

In August 1996 we studied an additional patient. She had no demonstrable toxicity to antibody infusion. We are currently in the process of evaluating tumor localization, radiation dose estimates, and pharmacokinetics. We are planning to study another eligible patient within the next month. -

Thus far, the results suggest that it is possible to administer therapeutic doses of radioimmunoconjugates to patients. The relatively low immunogenicity may allow for repeated administration. Within the next year, we plan to initiate a phase I radioimmunotherapy study using Y-90 MX DTPA huBrE-3. The study will involve both escalation of the amount of radioactivity and the number of administration in cohorts of

three patients each. Based on promising preclinical mouse experiments described below, we hope to initiate a phase I clinical trial with combination Y-90 MX DTPA huBrE-3 and continuous infusion topotecan.

Over the past year we expanded upon our initial observations demonstrating significant anti-tumor efficacy of continuous infusion topotecan in the mouse model and have performed experiments using combination topotecan infusion and Y-90 MX DTPA BrE-3.

In the fall of 1995, we performed biodistribution experiments in PLCR female mice. Twelve mice were given an intraperitoneal injection of 50 micrograms (0.1 cc) of murine BrE-3 containing 200 uCi of Y-90. Three mice each were sacrificed at 6 hours, 24 hours, 48 hours, and 72 hours post injection. The graph in Figure 1 (Appendix) outlines the % injected dose of 90-Y per gram of tissue for each organ. The majority of 90-Y activity was in liver, kidney, and bone at 48 hours post injection.

In late 1995, early 1996 we began combination studies in the mouse model. In the first experiment we used 7 groups of 3 Swiss nude mice each. Each mouse received a subcutaneous implantation (into left flank) of an 8 mm^3 chunk of the human mammary carcinoma MX-1 (approximately 1×10^7 cells). Fourteen days later, the mice were randomly assigned to a treatment arm, and, except for the control mice, had insertion of an Alzet pump containing either saline alone or topotecan at varying concentrations. The pumps allow for a continuous infusion of topotecan for 7 days. At day 8, the pumps were removed and replaced with new pumps containing the same amount of topotecan (saline for group 2) as the first pump for each group. The second pumps were removed on day 14. Thus, the 5 groups of mice that were randomized to topotecan, received the drug as a 14 day infusion. After the mice were anesthetized with IP Avertin, the pumps were implanted by a small incision under the skin in the right flank. After the placement of the pump, the skin was stapled and the wound was disinfected with betadine. In addition, groups 2, and 4-7 received an intraperitoneal injection of 50 micrograms of murine MX-DTPA BrE-3 labeled with 180 uCi of 90-Yttrium on day 0 (14 days post tumor implantation). The groups were randomly assigned as follows: (Appendix, Figure 2)

Group #	Alzet pump	Topotecan dose(per pump)	Y-90
1 (n=3)	none	0	0
2 (n=3)	yes	0	180uCi
3 (n=3)	yes	2mg/m^2	0
4 (n=3)	yes	0.2mg/m^2	180uCi
5 (n=3)	yes	0.5mg/m^2	180uCi
6 (n=3)	yes	1.0mg/m^2	180uCi
7 (n=3)	yes	2mg/m^2	180uCi

The mice were observed for 3 weeks post therapy. As demonstrated in Figure 2, the control mice had continued tumor growth over 21 days, as expected. The groups treated with 90-Y-MX-DTPA-BrE-3 monoclonal antibody or with topotecan alone had a small decrease in tumor growth relative to control. The groups receiving combination therapy had a much greater decrease in the growth rate of the tumor. The greatest effect was noted in group 6 when topotecan was given at 1.0 mg/m^2 per 7 days. At the higher dose of topotecan (2.0 mg/m2 over 7 days) in combination with 90-Y-BrE-3, all the mice died, presumably from drug toxicity.

In April 1996, we performed another experiment using greater numbers of mice per group. Again, Swiss nude mice were implanted with MX-1 tumors fourteen days prior to starting therapy as described above. Two groups of mice received topotecan infusion for 14 days starting day 0 via Alzet pump as described above. For this and all subsequent experiments the dose of topotecan used was 1.0 mg/m^2 over 7 days as this was the most efficacious in the previous experiment. The dose of murine 90-Y-MX-DTPA BrE-3 used in this experiment was between 180-185 uCi in 50 microgram of total protein (0.1cc). This was given as a bolus IP injection on day 0. The mice were randomly assigned to the following groups:

Group #	Alzet pump	Topotecan dose(per pump)	Y-90
1 (n=5)	no	0	0
2 (n=7)	yes	1.0mg/m^2	0
3 (n=7)	yes	0	180uCi
4 (n=7)	yes	1.0mg/m^2	185uCi

As noted in Figure 3 (Appendix), the control group had continued tumor growth. The group of mice treated with 90-Y-BrE-3 or topotecan alone had a decrease rate of growth relative to the control. The combined therapy group had a dramatic decrease in tumor weight which persisted for the duration of the experiment (3 weeks). Thus, we demonstrated in this experiment that the tumor burden decreased from baseline in the combined therapy group.

We then performed a confirmatory and slightly different experiment in the Swiss nude mice. In this experiment the mice were implanted with MX-1 tumor as described previously. The tumors were allowed to grow for 21 days before randomization for therapy. Alzet pumps were implanted into the mice on day 21 as described above. Two groups of mice received 200 uCi of 90-Y-BrE-3, 21 days after tumor implantation. Two groups of mice received a 14 day infusion of topotecan also beginning 21 days post tumor implantation. The groups were randomized as follows:

Group #	Alzet pump	Topotecan dose(per pump)	Y-90
1 (n=6)	no	0	0
2 (n=7)	yes	1 mg/m^2	0
3 (n=7)	yes	0	200uCi
4 (n=7)	yes	1 mg/m^2	200uCi

As demonstrated in Figure 4 (Appendix), the mice treated with combination topotecan and 90-Y-BrE-3 had complete disappearance of tumor xenograft for up to 50 days post tumor implantation. The mice in groups 2-4 will continue to be observed until tumor progression develops.

The mice experiments conducted thus far provide promising evidence for the synergistic effect of combination topotecan and murine 90-Y-Mx-DTPABrE-3 monoclonal antibody in the mouse human tumor xenograft model. In the next several months we plan to conduct additional experiments which will compare the aforementioned combination therapy with 90-Y tagged to a nonspecific antibody, and with the humanized form of BrE-3. In this manner we will be better able to extrapolate the data obtained in mouse model to phase I clinical trials where we plan to use the humanized BrE-3. We also want to document that the specificity of BrE-3 to the tumor is adding to the antitumor effect, thus 90-Y will be tagged to a nonspecific antibody and the antitumor effect will be compared to that of 90-Y-BrE-3. We also plan to initiate in vitro experiments using MCF-7 and other breast cancer cell lines in an attempt to determine the mechanism of action of tumor cell kill with combination topotecan and radioimmunoconjugates.

Conclusion

In conclusion, over the last year we have initiated a radioimaging study of 111In-Mx-DTPA-huBrE-3 in patients with metastatic breast cancer. We have treated 3 patients thus far. Preliminary results suggest that a therapeutic trial of 90-Y-Mx-DTPA huBrE-3 is feasible and repeated doses can be administered. In addition we have performed several experiments in the mouse model with combination radioimmunotherapy and continuous infusion topotecan. We have preliminary data demonstrating that the combination therapy eradicated the MX-1 implanted tumors in nude mice. Future studies in the mouse model will involve long term observation in the treated mice and possible repeat administration of combination therapy if tumor regrowth occurs. Toxicity studies will also be performed. In this manner, we hope to develop a phase I study using combined radioimmunotherapy and continuous infusion topotecan which will be of greatest benefit for women with metastatic breast cancer.

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APPENDIX

Publications

Appendix to Progress Report

Table 1: Blood pharmacokinetics

	Dose administered	T1/2 blood	AUC
01HBRDK	4.12 mCi	24.05 ± 4.5 hrs	1.91 ± 0.05 mCi •hrs
02HBRRL	2.99 mCi	α=23.3 ± 258.5 hrs	
		β=100.7± 793.8 hrs	6.32± 1.54 mCi•hrs

Table 2: Dose estimates based on biodistribution of In-111 MX-DTPA huBrE-3 (rads/mCi)

Indium-111

	01HBRDK	02HBRRL	average	S.D.
kidneys	1.99	2.73	2.36	0.52
liver	5.91	2.71	4.31	2.26
lung	1.01	0.81	0.91	0.14
ovaries	0.51	0.46	0.49	0.04
red marrow*	0.5	0.45	0.48	0.04
red marrow†	0.93	1.43	1.18	0.35
spleen	3.46	2.08	2.77	0.98
urinary bladder	0.75	0.37	0.56	0.27
whole body	0.621	0.48	0.55	0.10

Yttrium-90

	01HBRDK	02HBRRL	average	S.D.
kidneys	14.9	27.6	21.25	8.98
liver	46.9	19.3	33.10	19.52
lung	5.85	5.99	5.92	0.10
ovaries	1.5	1.45	1.48	0.04
red marrow*	2.02	1.9	1.96	0.08
red marrow†	10.6	21.8	15.05	6.29
spleen	37.4	19.5	28.45	12.66
urinary bladder	1.81	1.07	1.44	0.52
whole body	2.82	2.27	2.55	0.39

*based on blood

†based on regions of interest

FIGURE 1

Biodistribution of Y-90 Mab %ID/gm. tissue.

Legend:
- 6 hrs
- 24 hrs
- 48 hrs
- 72 hrs
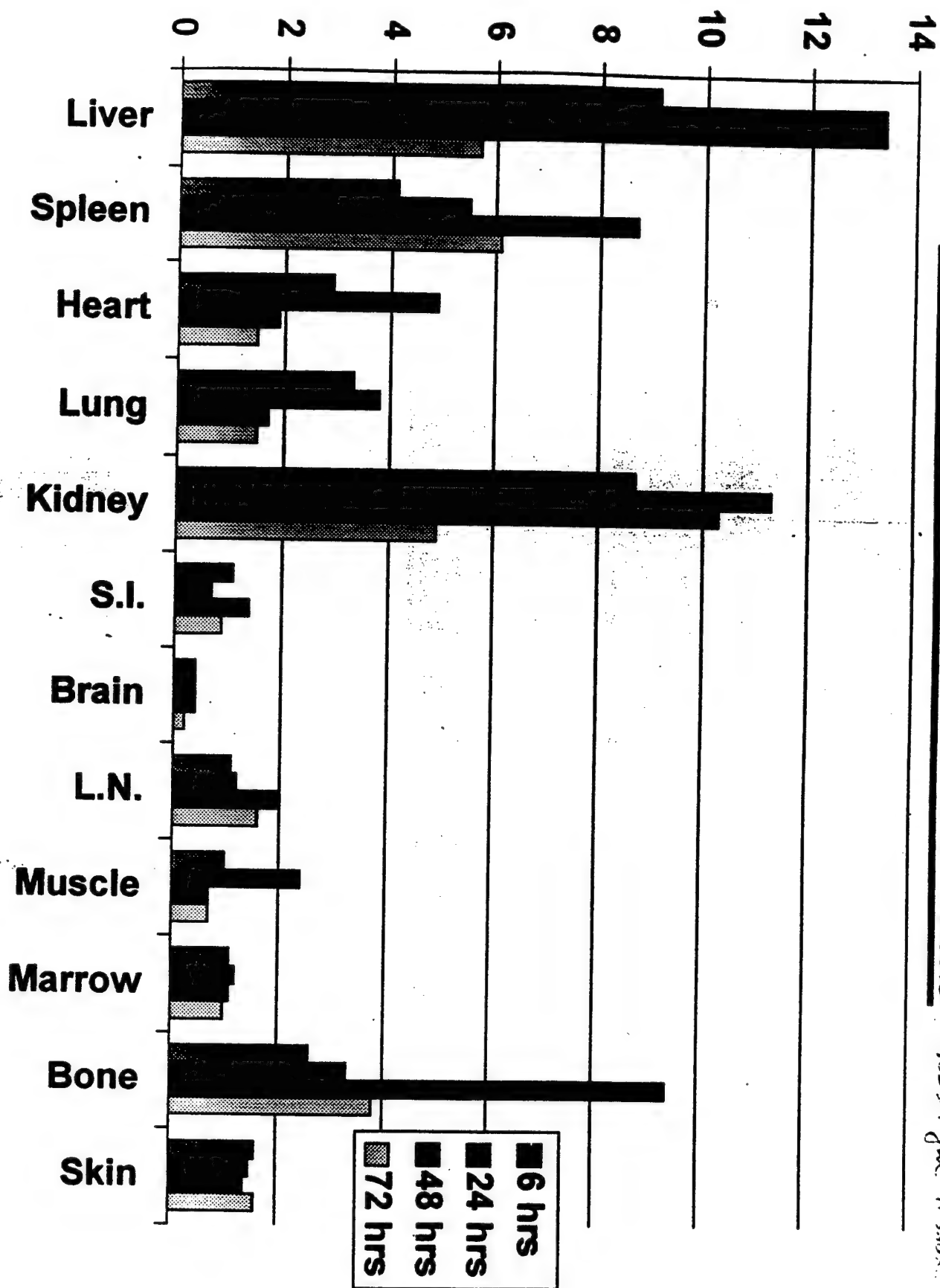
FIGURE 2

Topotecan and ^{90}Y Bre-3 MAb on Tumor Growth
Results: Tumor Volume 3/15/96

n=3 for each group

Legend:
- CTL
- 90Y (180uCi) only
- Topo I (2mg/m2) only
- 90Y + 0.2mg/m2 Topo I
- 90Y + 0.5mg/m2 Topo I
- 90Y + 1mg/m2 Topo I
- 90Y + 2mg/m2 Topo I
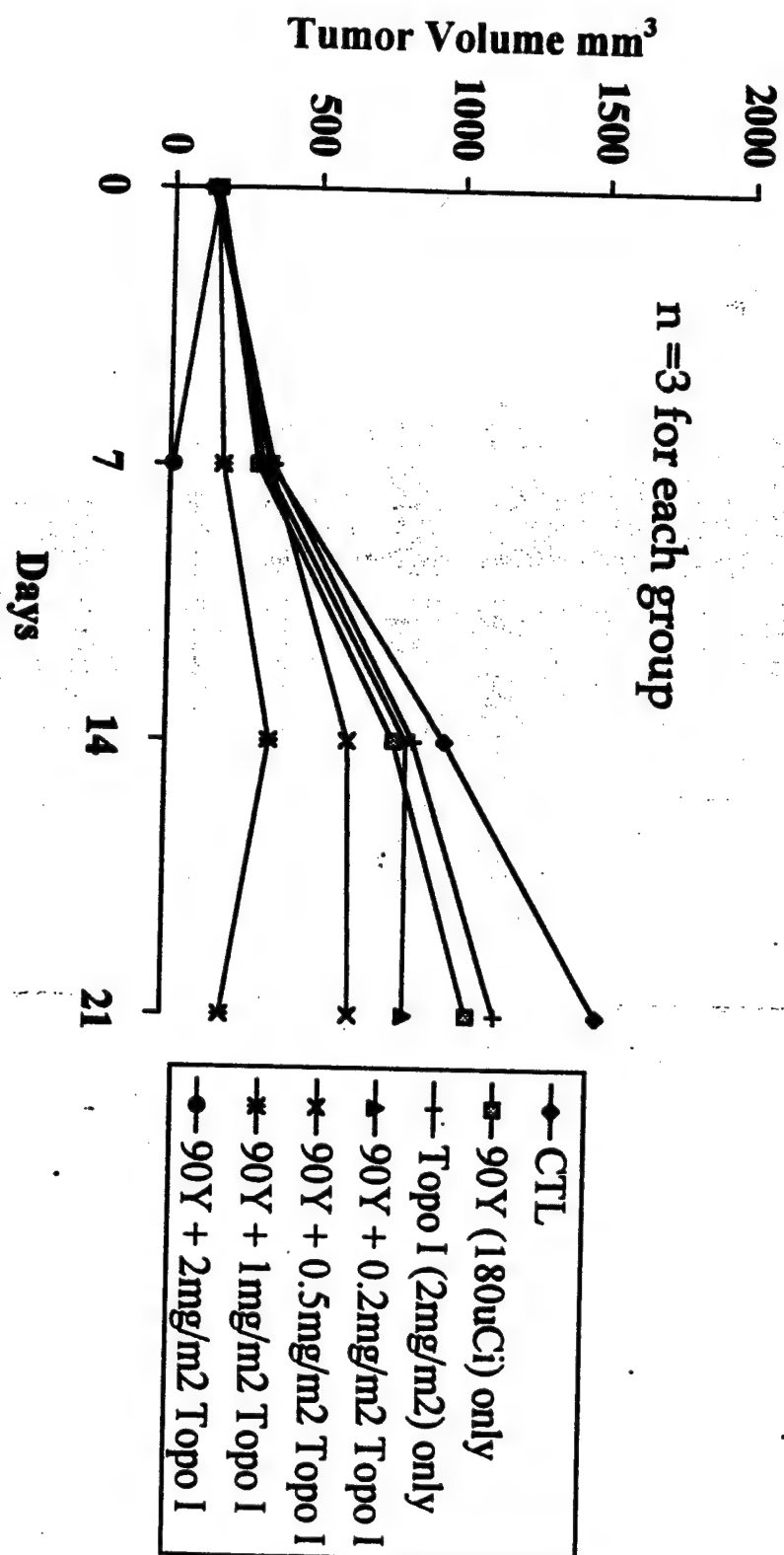
Effect of Topotecan and Bre-3 MAb 4/20/96
Results: Tumor Weights

FIGURE 3

91
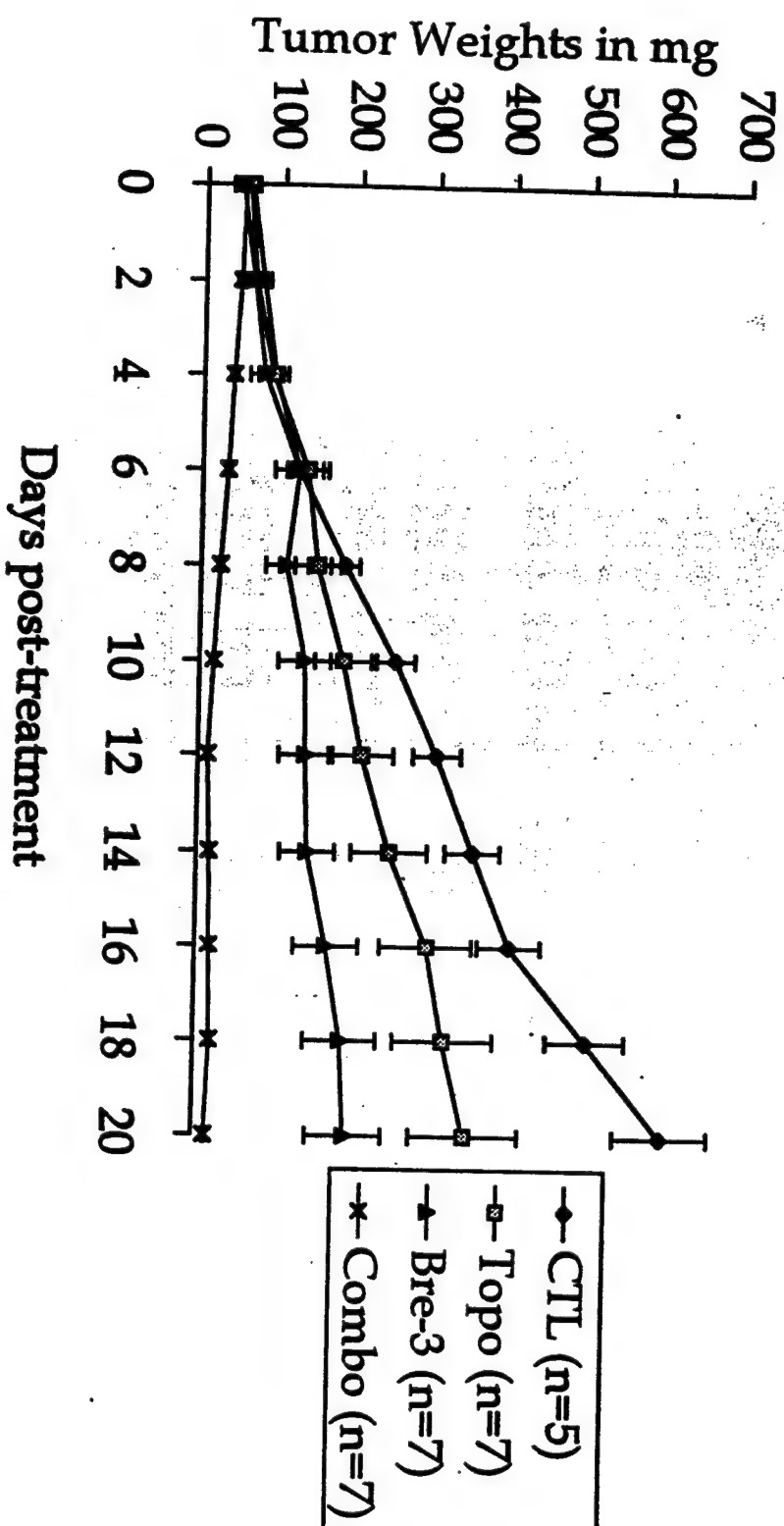
Effect of Topotecan (1mg/m²) and Bre-3 MAb (200uCi) 6/11/96

Results: Tumor Weights
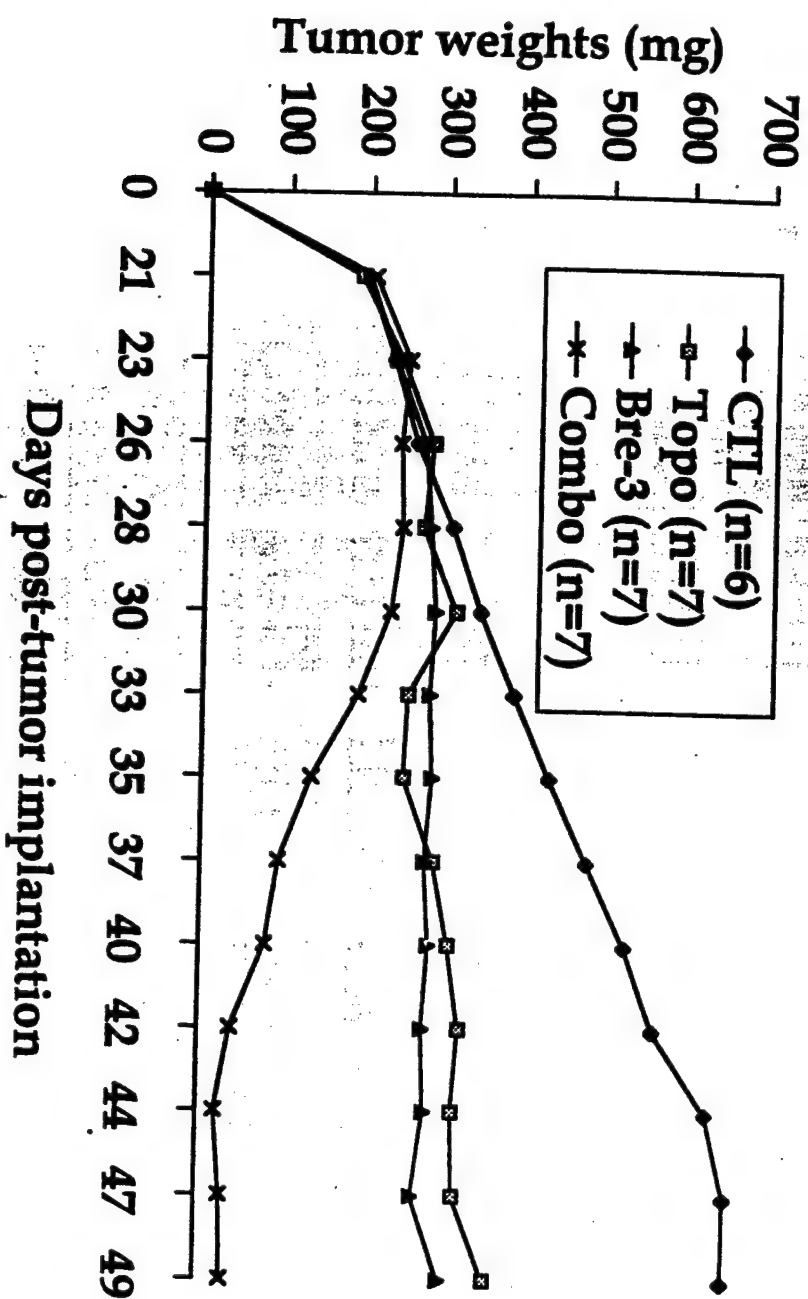
FIGURE 4

Tumor weights (mg)

- CTL (n=6)
- Topo (n=7)
- Bre-3 (n=7)
- Combo (n=7)

Days post-tumor implantation

AD_____

GRANT NUMBER DAMD17-94-J-4176

TITLE: Radioimmunotherapy of Metastatic Breast Cancer Using
         Radiolabeled Tumor Specific Antibodies

PRINCIPAL INVESTIGATOR: Carolyn Wasserheit, M.D.

CONTRACTING ORGANIZATION: New York University Medical Center
                            New York, New York   10010-2598

REPORT DATE: September 1997

TYPE OF REPORT: Annual

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designated by other documentation.

Introduction

The following is a progress report for Grant No. DAMD17-94-J-4176 entitled, "Radioimmunotherapy of Metastatic Breast Cancer Using Radiolabeled Tumor Specific Antibodies" for the period of September 1, 1996 to August 31, 1997.

The overall goal of the project is to develop effective therapy for breast cancer using radioimmunoconjugates. As noted previously, the humanized version of BrE-3 (huBrE-3) designed at the Cancer Research Fund of Contra Costa by Drs. Joseph Couto and Roberto Ceriani in collaboration with E. Padlan (NIH) has become available to us. The purpose of the design was to diminish the immunogenicity of the frameworks while strictly maintaining the antigen binding affinity.

A strategy aimed at increasing the potential efficacy of radioimmunotherapy is by combining this with other agents such as chemotherapy. As noted in my previous progress report, at NYU, a model drug that we have helped develop is the topoisomerase-1 inhibitor topotecan. Topoisomerase-1 is a nuclear enzyme involved in unwinding of supercoiled DNA and is integrally involved in a host of cell functions including replication and transcription. In the presence of topotecan, there is a stabilization of the complex formed by topoisomerase I and DNA, preventing the religation of the DNA strand. Interaction between the stabilized terniary complex and the replication fork is thought to convert single strand breaks into double strand breaks and cause cell death. Topotecan interaction with this enzyme converts topoisomerase-1 into a "cellular poison" and results in progressive cell death.

We have conducted a Phase I study utilizing a novel schedule for administration of topotecan, under sponsorship of CTEP. In this study topotecan was given as an ambulatory infusion in low doses, continuously for up to a 21-day duration. We have determined the MTD for heavily pretreated patients to be 0.53 mg/m2/day for 21 days, increasing dose intensity by >50% compared to the conventional (daily x 5) administration schedule. We have also observed unprecedented activity in a phase I study, including partial remissions in patients with ovarian and breast cancer (previously received 4-5 regimens) and renal cancer (1). We are currently completing a phase I study evaluating 3 hour paclitaxel and 14 day continuous-infusion topotecan in patients with advanced cancer.

While studies of topo-1 inhibitors in combination with radioimmunotherapy have not yet been reported, experimental models with external beam radiation therapy show that the combination of these two modalities enhance cell kill in cell culture and *in vivo* (2-6). It has been postulated that the synergism between the topo-1 inhibitors and ionizing radiation is due to the ability of topo-1 inhibitors to interfere with repair of radiation-induced DNA damage (7). Ionizing radiation sensitizes cells to topo-1 inhibitors by slowing their progression through S-phase, thus, increasing the number of cells in S-phase (4). The most optimal effects *in vivo* have been seen when the topo-1 inhibitor is given shortly before the irradiation (5), or concurrently with continuous application (8, 9).

While external beam irradiation of loco-regional disease is possible in a disease like primary non-small cell lung cancer, this is a less feasible approach with respect to metastatic breast cancer which may be more widely disseminated. Radioimmunoconjugates provide a vehicle for targeting therapeutic doses of radiation to widely dispersed tumor throughout the body. Similarly, the above principles of synergy will apply to radiation delivered by this method as well as by external beam, but with improved therapeutic index.

As previously reported, we have demonstrated the feasibility of administering topotecan as a continuous infusion in the mouse model. We also demonstrated an antitumor effect. As described below we have continued with our experiments using continuous infusion topotecan and radioimmunoconjugate. The data generated thus far support the use of combination therapy for future clinical trials.

Body

Over the last year, we have completed a phase I study using ^{111}In-MX-DTPA huBrE-3 in patients with advanced breast cancer (previously submitted to you.). To date, 87 patients have been referred for screening of tissue for BrE-3. Tissue blocks were available in 58. Of these, 35 patients' tissue demonstrated staining of > 25% of the cells in the tumor. Fourteen of our patients have had only fine needle aspiration (FNA) which we previously were unable to stain. With a new fixing technique, we are currently able to stain FNA specimens and in two patients we have obtained positive results.

We studied 7 patients on this protocol. They each received 2 mg of MX-DTPA huBrE-3 labeled with about 5mCi of Indium ^{111} plus 48 mg of nonconjugated BrE-3 intravenously over one hour. The antibody infusions were well tolerated. No allergic or toxic side effects were observed. One patient complained of a transient strange taste in her mouth. One patient developed grade 3 thrombocytopenia at 9 days after infusion of antibody. She was concurrently receiving external beam radiation to the spine and had extensive involvement of the bone marrow with metastatic carcinoma documented by bone marrow biopsy. Serologic analysis revealed no evidence of immunologic platelet destruction and it was felt that the thrombocytopenia was predominantly secondary to the combination of poor bone marrow reserve and external beam radiation and unlikely to be related to toxicity from the ^{111}In MX-DTPA huBrE-3. Patients underwent serial whole body counting, gamma camera imaging, plasma and urine sampling over one week in order to assess pharmacokinetics, radiation dose, tumor localization and pharmacokinetics. We imaged 76% of known bone, liver, and lung lesions and identified two sites previously unsuspected (lymph node, bone). Blood pharmacokinetics show a longer half-life for the humanized antibody than for the murine. In six patients the $T1/2_{alpha}$ for the humanized antibody averaged 106.5±8.5 hours and the $T1/2_{beta}$ averaged 114.2±39.2 hours. Radiation dose estimates for (using standard MIRD formalism) have been made for normal organ and tumor. Dose estimates to tumors averaged 82± 22 rads/mCi administered for ^{90}Y-MX-DTPA huBrE-

3 with average marrow dose estimated at 5±3 rads/mCi administered (see appendix tables 1 and 2). Immunogenicity has now been studied out to 3 months in 3 of our patients. Qualitative analysis of serum incubated with either radioiodinated huBrE-3 or Indium-111 labeled hu BrE-3 demonstrates that compared to baseline serum, there are trace amounts of antibody-antibody formation at 5 weeks and 3 months after antibody infusion. Since these anti-humanized antibodies react equally with the murine BrE-3, we believe that "HAHA" represents an idiotypic response.

Thus far, the results suggest that it is possible to administer therapeutic doses of radioimmunoconjugates to patients. The relatively low immunogenicity may allow for repeated administration. We have just closed the radioimaging study and have had recent IRB approval of a phase I radiotherapeutic study using dose fractionated Y-90 MX-DTPA huBrE-3 in patients with advanced breast cancer (protocol in appendix). We are in the process of screening patients who are potential candidates for this study. We ultimately plan to proceed with a combination phase I trial of radioimmunotherapy and topoisomerase I inhibitor therapy based on the promising results of our preclinical work previously described and discussed below.

Over the past year we expanded upon our initial observations demonstrating significant anti-tumor efficacy of combination continuous infusion topotecan and Y-90 MX-DTPA BrE-3 in the mouse model. As previously described, we conducted a series of experiments in the athymic female nude mouse model implanted with the human transplantable breast tumor line MX-1 in the left flank. In the last experiment previously reported, on day 21 after implantation of MX-1, mice were randomized into four treatment groups: Control (no treatment), BrE-3 (i.p. 50 ug of murine MX-DTPA BrE-3 labeled with 200uCi of ^{90}Yttrium), topotecan (1mg/m2 for 14 days via s.c. Alzet pump), and combination (BrE-3 and topotecan). Body weights and tumor weights were measured every 3-4 days. As noted in Figure 1, the control mice all died by day 69 after tumor implantation. In the groups treated with BrE-3 or topotecan alone, the mice had reduced tumor growth for about 50 days post treatment but then the tumor grew to sizes comparable to the untreated tumor bearing mice. The mice that received the combination therapy had a substantial decrease in tumor cell growth that resulted in compete tumor regression in 10 of 13 mice. At sacrifice 120 days after treatment, none of the surviving 10 mice had any sign of recurrent tumor.

Our next experiment was performed in order to determine whether the observed tumor response is primarily due to the specificity of the 90Y-MX-DTPA-BrE-3 or to the systemic circulating level of radioactivity given to the animal. A non-specific, isotype matched-matched monoclonal antibody(MOPC) was used for the combined therapy. In addition, the humanized BrE-3 was substituted for the murine antibody as the humanized BrE-3 is used in clinical trial as previously described. Athymic female nude Swiss NIH mice were implanted with human mammary carcinoma (MX-1). On day 21, mice were randomized into one of 5 groups as follows:

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Group #	hu-90-Y-BrE-3	90Y-MOPC	Topo (1mg/m2 x14days)
1 (n=6)	-	-	-
2 (n=6)	+	-	-
3 (n=6)	-	+	-
4 (n=6)	+	-	+
5 (n=6)	-	+	+

As shown in Figure 2, their was no significant tumor inhibition noted in group #3 and their was no difference in survival between the control group and group #3. The synergistic effect noted with the combined 90Y-MX-DTPA BrE-3 and Topo was not observed in the combined MOPC and topotecan group. Only a transient inhibition of tumor growth was noted in group #5 which was similar in effect and survival to the group treated with BrE-3 alone. The huBrE-3 in combination with topotecan demonstrated the same synergism as with the murine antibody.

Our next experiment was performed to evaluate treatment efficacy and morbidity in animals treated with a single dose of 200uCi 90Y labeled MX-DTPA BrE-3 compared to two fractionated doses of 90Y labeled MX-DTPA BrE-3 in combination with topotecan. On day 21 post tumor implantation, mice bearing MX-1 tumors were injected either with a single dose of 200uCi 90Y labeled with MX-DTPA BrE-3 or with 2 weekly injections of 125uCi 90Y labeled with MX-DTPA BrE-3. Mice in each group were then randomized to receive topotecan (1mg/kg for 14 days) via Alzet pump as follows:

Group #	200uCI BrE-3 + Topo	125uCi BrE-3x2	125uCiBrE-3 x2+Topo
1(n=6)	-	-	-
2(n=6)	+	-	-
3(n=6)	-	+	-
4(n=6)	-	-	+

As shown in Figure 3, both combination groups #2 and #4 had dramatically reduced tumor growth which were of the same magnitude in the fractionated 90Y labeled dosing as in the single dose.

In order to help elucidate the cell-damage mechanism of the combined therapy, several *in vitro* experiments were performed to determine the effect of topotecan, 90Y labeled BrE-3, and the combination on cell proliferation. Two tumor cell lines were utilized for these *in vitro* studies, the MDA-MB 157 (BrE-3 positive) and the MDA-MB 435 (BrE-3 negative) human mammary carcinoma's. Colorimetric (MTT) assay is used to determine cell survival and proliferation. MTT(3-(4,5-dimethylthiazol-2-yi)-2,5 diphenyl tetrasolium bromide) is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria. In this experiment, $1x10^4$ cells are plated in 96 wells plate overnight in DMEM media with

10% FBS. 90Y-BrE-3 (5ng to 500ng Ab proteins) are added to half of the wells and incubated for 1 hour. Cells were washed three times to remove all the unbound 90Y-BrE-3 antibodies. Escalating concentrations of topotecan (0uM to 10uM) were then added to the appropriate wells. Cells incubated in media alone served as controls. Plates were incubated for four days and MTT were then added to the wells and allowed to incubate for an additional four hours. Cells were lysed with HCL/isopropanolol and absorbance measured on an ELISA plate reader with a test wavelength of 570 nm and a reference wavelength of 630 nm. BrE-3 binding affinity is ten-fold higher in the MDA-MB 157 than in MDA-MB435. In both cell lines, topotecan exerts a dose escalating reduction of cell proliferation rate (see figure 4). However, in the presence of 90-Y monoclonal Abs, only the MDA-MB 157 cell line which retained 90-Y BrE-3 exhibited a further decrease in cell proliferation rate (data not shown).

In addition, an *in vitro* assay was established with the MDA-MB 157 cell lines and the MDA-MB 435 cell lines described above. Similar to the last experiment, 1×10^4 cells are plated in 96 wells overnight in DMEM media with 10% FBS. 90Y BrE-3 (5 ng to 500 ng Ab proteins) were added to half of the wells and incubated for 1 hour. Cells were then washed three times to remove all the unbound 90Y BrE-3 antibodies. Escalating concentrations of topotecan (0uM to 10uM) were then added to the appropriate wells. At the end of 3 days incubation, cells were lysed and supernatant collected for determining rate of apoptosis. The assay used to measure the degree of apoptosis (Cell Death Detection ELISA, Boehringer Mannheim) is based on the quantitative sandwich-enzyme-immunoassay-principle using a mouse monoclonal antibodies directed against DNA and histones, respectively. This allows the specific determination of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. Briefly, anti-histone antibody is fixed absorptively on the wall of the microtiter plate. Supernatant which contained the cytoplasmic fraction of cells were added to the wells and allowed to incubate at room temperature for 90 minutes. After several washings, anti-DNA-peroxidase conjugating solution was added to the wells for an additional 90 minutes incubation at room temperature. Substrate solution was added for photometric analysis and absorbance was measured on an ELISA plate read with a test wavelength of 405nm and a reference wavelength of 490nm. As shown in figures 5 and 6, topotecan is able to induce and increase of apoptosis in a dose escalating manner for both MDA-MB157 and MDA-MB435 cell lines. No additive increase is seen in MDA-MB435 cells treated with 90-Y BrE-3 monoclonal antibody. For the MDA-MB157 cells, however, their is an increased rate of apoptosis in the presence of BrE-3 alone and an additive effect is demonstrated when both topotecan and BrE-3 are administered in combination.

Conclusion

In conclusion, over the last year we have completed a radioimaging study of ^{111}In-Mx-DTPA-huBrE-3 in patients with metastatic breast cancer. We are in the process of preparing a manuscript for publication. The results suggest that a therapeutic trial of 90-Y-Mx-DTPA huBrE-3 is feasible and repeated doses can be administered. We have recently opened a therapeutic phase I study described above. In addition we have continued to perform our experiments in the mouse model with combination

radioimmunotherapy and continuous infusion topotecan. As previously noted, the experiments demonstrated that topotecan enhances the therapeutic index of radioimmunotherapy against human mammary carcinoma. Substituting a nonspecific MOPC antibody for BrE-3 did not result in an anti-tumor response indicating that the specific binding of the BrE-3 antibody to the tumor is necessary. In addition, substituting murine BrE-3 for humanized BrE-3 resulted in similar antitumor efficacy. Using a fractionated dose of BrE-3 given two times resulted in a similar anti-tumor effect to one dose of BrE-3. Our *in vitro* studies demonstrated a decrease in cell proliferation rate and an increase in the apoptotic rate in the cells treated with topotecan. The MDA-MB 157 cells which are BrE-3 +, demonstrated an additive effect on the apoptotic rate when treated with a combination of BrE-3 and topotecan. The data generated thus far continue to support the rationale for using this combined modality therapy in women with advanced breast cancer. In the upcoming year we plan to complete our phase I study of fractionated 90Y-BrE-3 and to initiate a phase I study of combined radioimmunotherapy and continuous infusion topotecan in women with advanced breast cancer. In our preclinical experiments, we will investigate other chemotherapeutic agents such as paclitaxel and gemcitabine in combination with radioimmunotherapy. If this or other agents reveal synergistic activity we will pursue studying these combinations in patients.

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APPENDIX

Table 1:   Dose estimates based on biodistribution of In-111 MX-DTPA huBrE-3
              (rads/mCi) for Indium-111
Table 2:   Dose estimates based on biodistribution of In-111 MX-DTPA huBrE-3
              (rads/mCi) for Yttrium-90

Figure 1:   Effect of Combined Treatment of Topotecan & BrE-3 on Breast Cancer
              Xenografts
Figure 2:   MOPC isotype (180uCi) and humanized BrE-3 (180uCi) Tumor Weights
              10/22/96
Figure 3:   Effect of Fractionated BrE-3 on Breast Tumor Xenografts
Figure 4:   MTT Cell Proliferation: MDA-MB435 (BrE-3 negative)
Figure 5:   Apoptosis:MDA-MB435 (BrE-3 negative)
Figure 6:   Apoptosis: MDA-MB157 (BrE-3 positive)

Protocol: A Phase I study of the Toxicity and Dosimetry of a humanized Breast-
              Directed Monoclonal Antibody (BrE-3) radiolabeled with [111]Indium
              (^{111}In) and [90]Yttrium (^{90}Y)

Dose estimates based on biodistribution of In-111 MX-DTPA huBrE-3 (rads/mCi)

Table 1

Indium-111
(rads/mCi)

	01HBR	02HBR	03HBR	04HBR	05HBR	average	S.D.
kidneys	1.99	2.73	1.68	3.22	2.41	2.41	0.61
liver	5.91	2.71	3.03	2.17	2.03	3.17	1.58
lung	1.01	0.81	0.83	1.34	1.33	1.06	0.26
ovaries	0.51	0.46	0.41	0.52	0.56	0.49	0.06
red marrow*	0.5	0.45	0.51	0.66	2.09	0.84	0.70
spleen	3.46	2.08	1.69	1.9	1.74	2.17	0.73
urinary bladder	0.75	0.37	0.34	0.42	0.38	0.45	0.17
whole body	0.621	0.48	0.45	0.52	0.54	0.52	0.07

Table 2

Yttrium-90
(rads/mCi)

	01HBR	02HBR	03HBR	04HBR	05HBR	average	S.D.
kidneys	14.9	27.6	14.6	33.8	23	22.78	8.27
liver	46.9†	19.3†	22.2†	14.7	13.4	23.30	13.66
lung	5.85	5.99	6.39	13.7††	13.2	9.03	4.05
ovaries	1.5	1.45	1.27	1.62	1.26	1.42	0.15
red marrow*	2.02	1.9	3.89	5.69	9.85	4.67	3.29
spleen	37.4	19.5	16.10	16.6	15.2	20.96	9.33
urinary bladder	1.81	1.07	1.09	1.31	0.94	1.24	0.34
whole body	2.82	2.27	2.01	2.35	2.38	2.37	0.29
tumors	101.35	89.63	92.8/81.4	**	43.7	81.78	22.45

*based on blood

†Patients 2 and 3 had liver metastases. Patient 1 manifested diffuse metastases on CT within 3 months.

†† multiple lung metastases

**No measurable tumors visualized on planar images. Dosimetry could not be calculated.

Figure 1

Effect of Combined Treatment of Topotecan & BrE-3
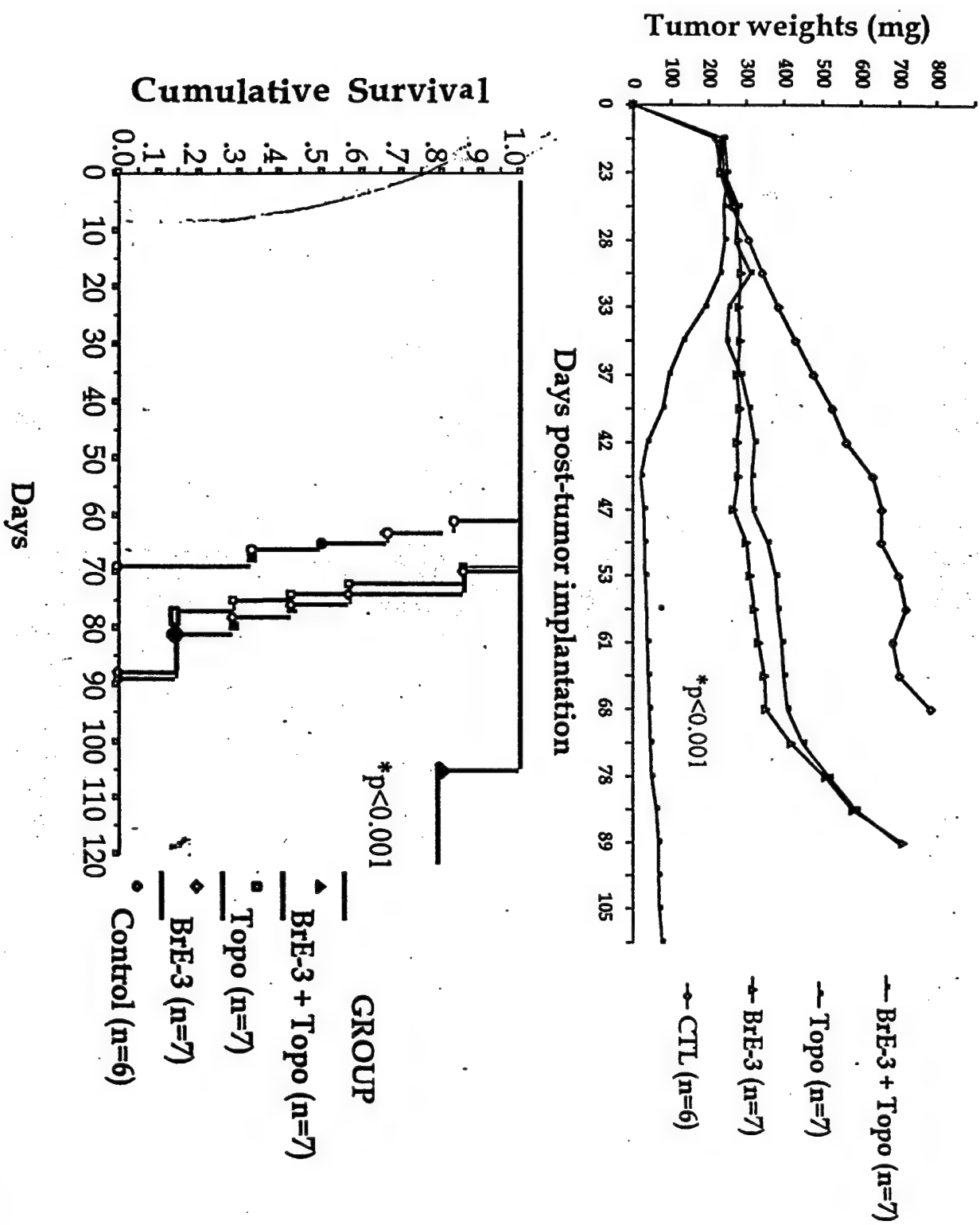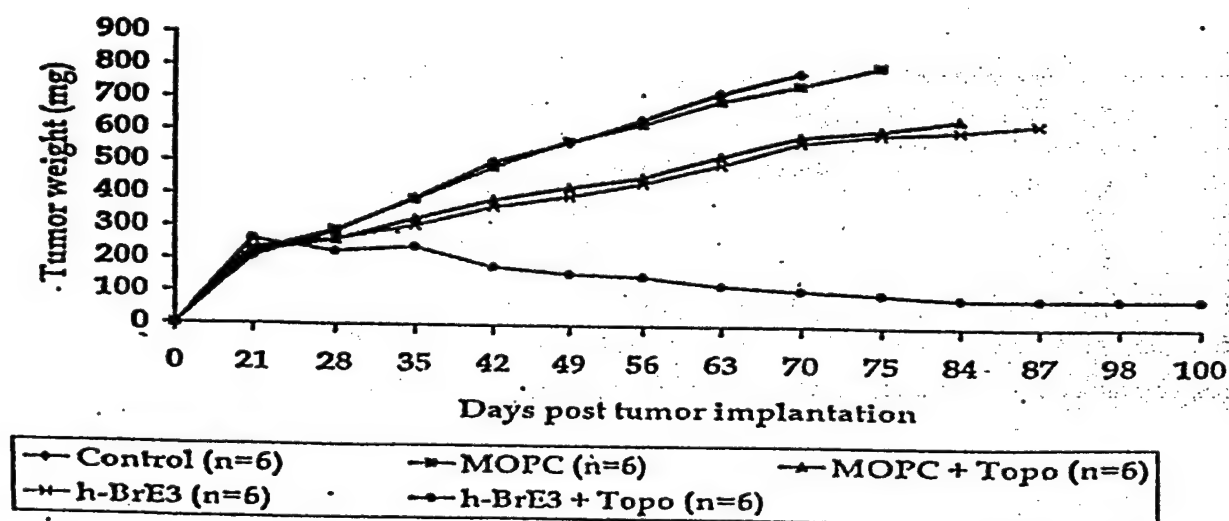on Breast Cancer Xenografts

Figure 2

MOPC isotype (180uCi) and humanized BrE3 (180 uCi) Tumor Weights 10/22/96

Effects of combination treatment with humanized BrE3 and non-specific radiolabeled antibody (MOPC). The MOPC alone was comparable to the not treatment animal group, while the combination MOPC/topotecan was comparable to the topotecan treatmetn group alone. The combination HuBrE-3 topotecan showed the same syngerestic activity observed with the murine BrE-3 antibody.

Figure 3: Effect of Fractionated BrE-3 on Breast Tumor Xenografts

Tumor Weights (mg)

Days post-tumor implantation

- CTL (n=6)
- 200uCi BrE-3 + Topo (n=6)
- 125uCi x 2 Bre-3 (n=6)
- 125uCi x 2 Bre-3 + Topo (n=6)

Figure 4

MTT Cell Proliferation: MDA-MB 435 (BrE-3 negative)

[90-Y BrE-3]

OD 570-630

Legend:
- 0uM Topotecan
- 0.1uM Topotecan
- 1uM Topotecan
- 10uM Topotecan

Apoptosis: MDA-MB 435 (BrE-3 negative)

Figure 5

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Figure 6

Apoptosis: MDA-MB 157 BrE-3 positive

[90-Y BrE-3]

OD 405-490

Legend:
- 0uM Topotecan
- 0.1uM Topotecan
- 1uM Topotecan
- 10uM Topotecan
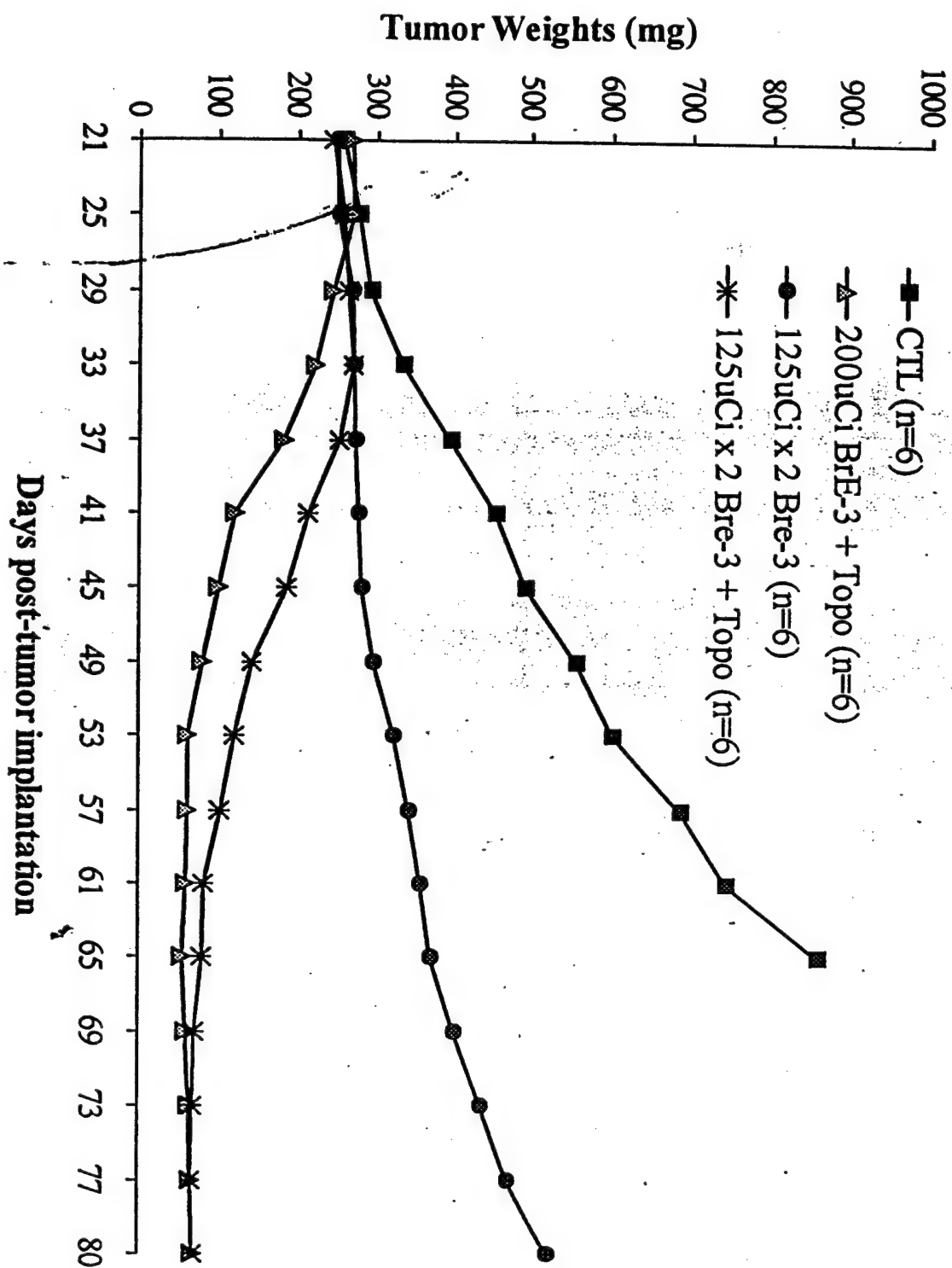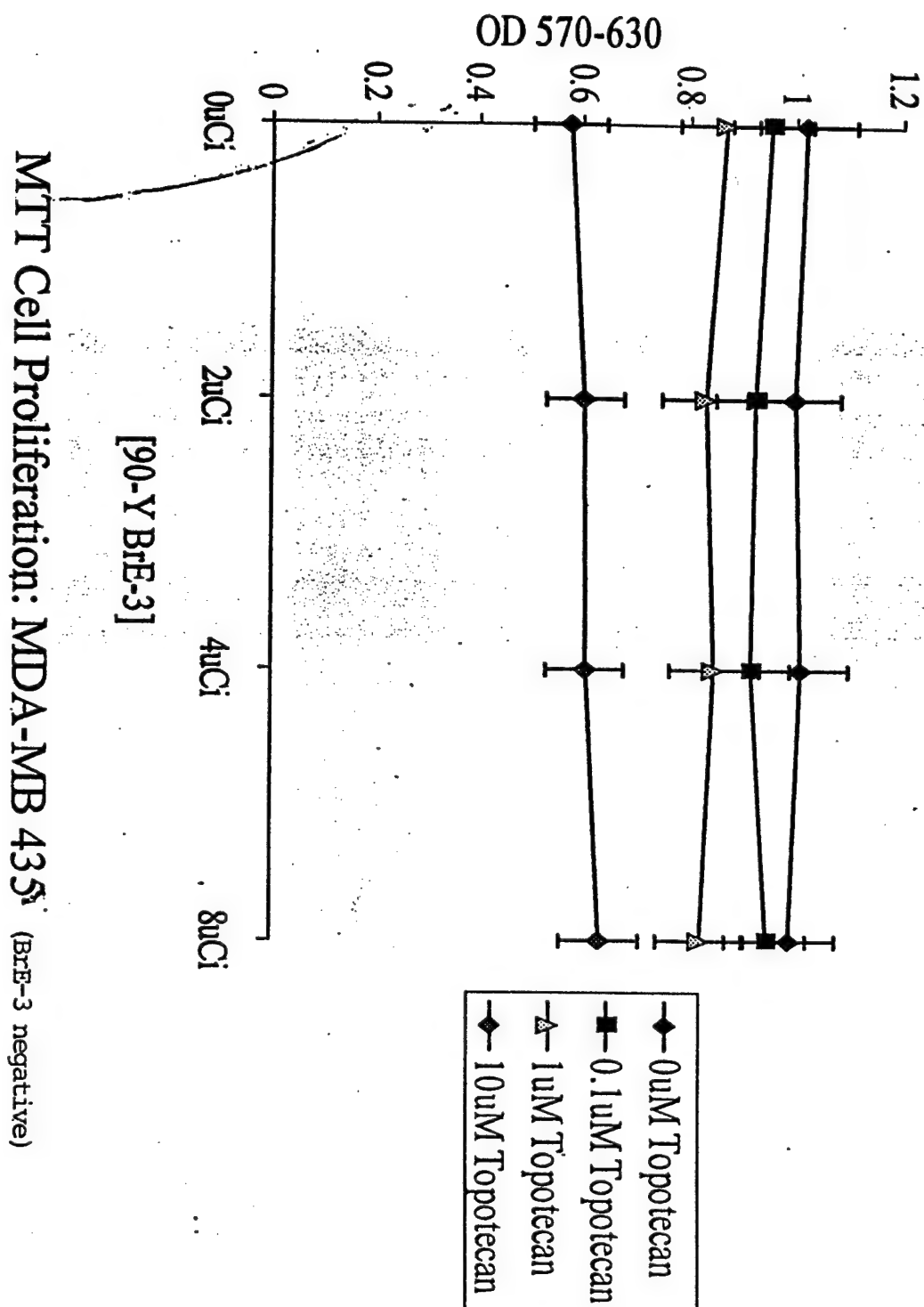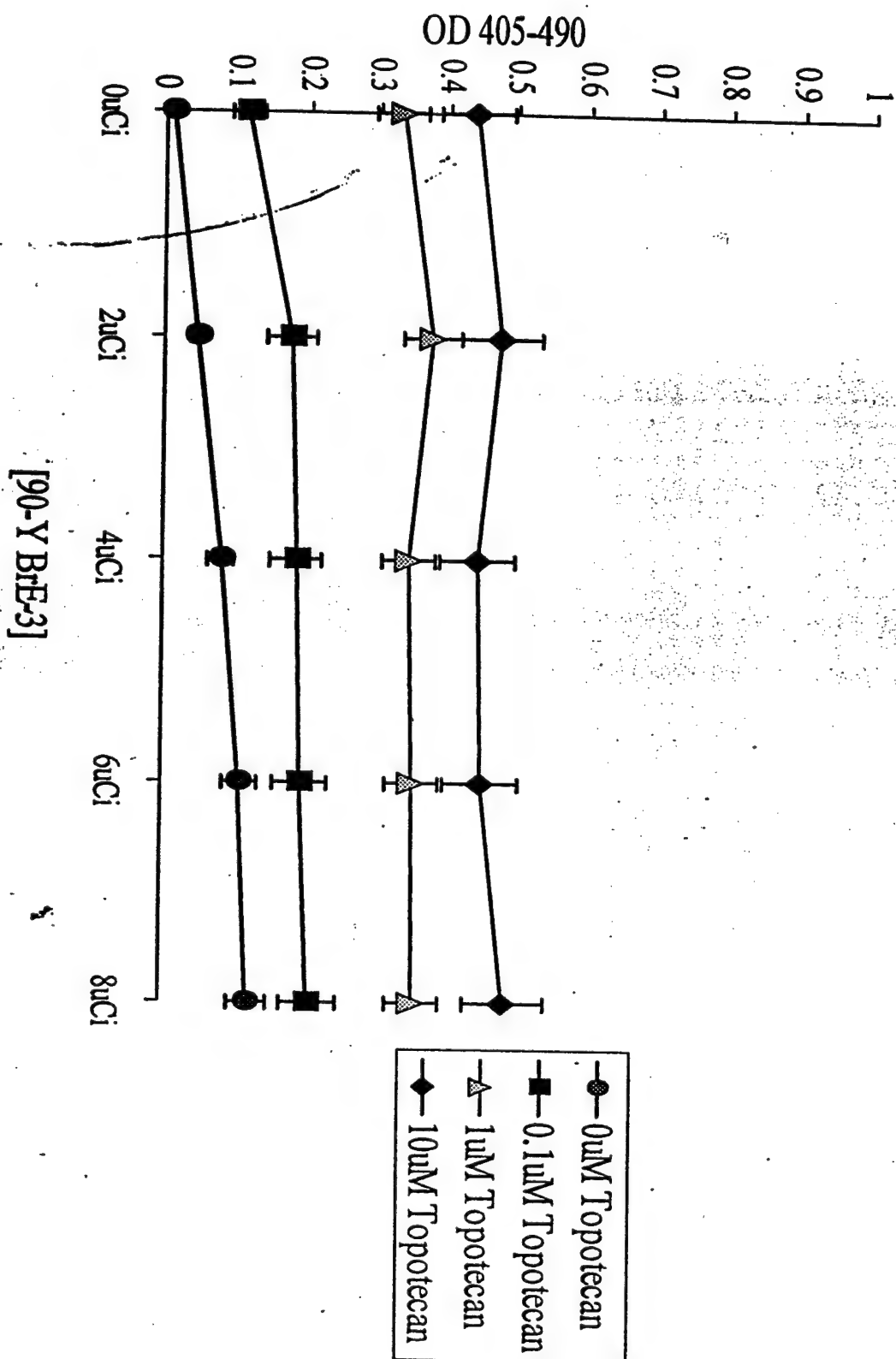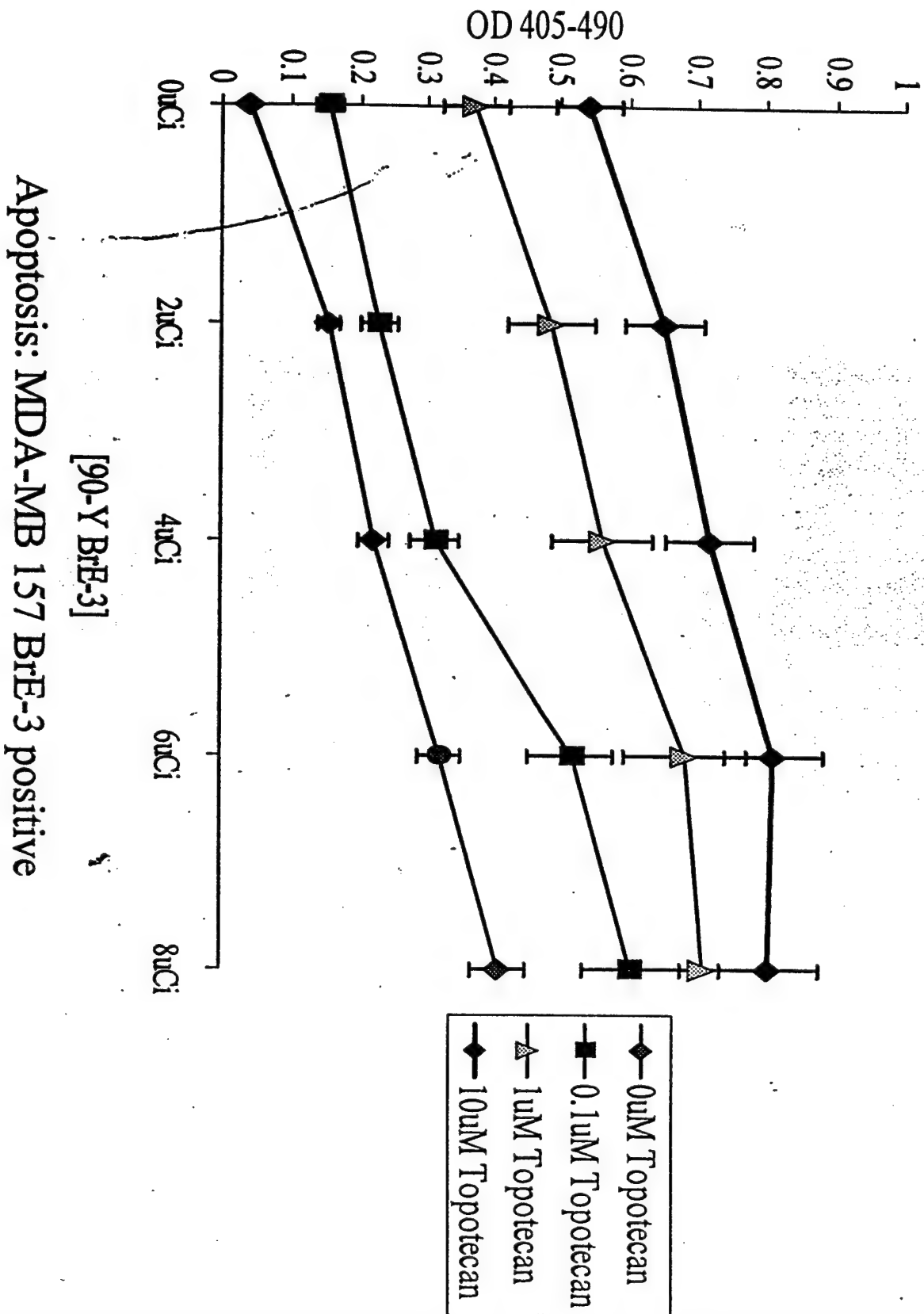
Publications

1. Paper

Kramer EL, Liebes L, Wasserheit C, Noz ME, Blank EW, Zabalegui A, Melamed J, Furmanski P, Peterson JA, Ceriani RL. Initial Clinical Evaluation of Radiolabeled MX-DTPA Humanized BrE-3 Antibody in Patients with Advanced Breast Cancer. Clin Can Res. 1998, 4: p1679-1688

2. Abstracts

Wasserheit C, Kramer EL, Liebes L, Noz M, Zabalegui A, Rios E, Fry D, Blank E, Sanger J, Ceriani R, Peterson J , Furmanski P. Initial Studies in Patients with Advanced Breast Cancer Using In-111 MX-DTPA Humanized BrE-3 Monoclonal Antibody. Presented at Sixth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer: 1996, Princeton, NJ

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Initial Clinical Evaluation of Radiolabeled MX-DTPA Humanized BrE-3 Antibody in Patients with Advanced Breast Cancer[1]

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ABSTRACT

To evaluate radiometal-labeled humanized BrE-3 (huBrE-3) monoclonal antibody as a radioimmunolocalization and therapeutic agent in breast cancer patients, tumor localization, pharmacokinetics, radiation dosimetry, and immunogenicity of ^{111}In-labeled combined 1-p-isothiocyanatobenzyl 3-methyl- and 1-p-isothiocyanatobenzyl 4-methyl-diethylenetriamine pentaacetic acid (MX-DTPA) huBrE-3 were studied. Seven women with BrE-3 antigen-positive, metastatic breast carcinoma underwent ^{111}In huBrE-3 infusion (5 mCi; 50 mg), followed by serial gamma camera imaging and plasma sampling. Region of interest analysis of images was used to make radiation absorbed dose estimates for ^{111}In huBrE-3. Data were extrapolated to ^{90}Y huBrE-3. Human anti-human antibody (HAHA) response was measured in serum samples obtained up to 3 months after infusion. Patients tolerated infusions well. Seventy-six percent of 105 known sites of disease were identified on planar and single-photon emission computed tomography scans. For six of seven patients, a biexponential model fit the plasma time-activity curve best with an average $T_{1/2\alpha} = 10.6 \pm 8.5$ (SD) h and average $T_{1/2\beta} = 114.2 \pm 39.2$ h. Radiation absorbed dose estimates for ^{111}In huBrE-3 for whole body averaged $0.53 \pm .08$ rads/mCi. Dose estimates for ^{90}Y huBrE-3 for marrow averaged 8.4 ± 11.9 rads/mCi, and for tumors, 70 ± 31.5 rads/mCi. Liver radioactivity uptake averaged 19.7 $\pm$ 8.8% injected dose at 24 h after infusion, translating into an average radiation absorbed dose 21.1 $\pm$ 12 rads/^{90}Y mCi administered. Only one of seven patients demonstrated a low level of HAHA response. Although the plasma half-lives are longer and marrow dose higher for radiolabeled huBrE-3 compared with the murine construct, the excellent tumor localization, good tumor dosimetry, and low immunogenicity support the use of ^{90}Y-huBrE-3 antibody for radioimmunotherapy of breast cancer.

INTRODUCTION

Breast cancer remains the second most common cause of cancer deaths in women in the United States today. Once a patient recurs with metastases, therapy will prolong but not improve overall survival from this disease. Response rates to second-line therapies are at best 50% and often lower. New approaches to the treatment of advanced breast cancer like radioimmunotherapy, using radiolabeled tumor-directed antibodies, hold promise for improving this prognosis.

One antibody that has shown potential in radioimmunotherapy of breast cancer is BrE-3 labeled with ^{90}Y. The BrE-3 antibody targets the epitope of the M_r 400,000 breast epithelial mucin. The epitope, which results from abnormal glycosylation of mucin in tumors, is highly abundant and prevalent in breast carcinoma (1). Radioimmunolocalization trials with ^{111}In-MX-DTPA murine BrE-3 have shown excellent tumor localization (2). Radioimmunotherapy with ^{90}Y-MX-DTPA murine BrE-3 has yielded objective minimal and transient responses at low doses in three of six patients (3) and partial responses in four of eight patients at high doses (4). In spite of these responses, however, retreatment has been obviated by the presence of a human anti-murine antibody response in a majority of these patients (3, 5).

Immunogenicity has proved a persistent stumbling block in radioimmunotherapy in many trials using murine immunoconjugates (6–11). Genetic engineering of antibodies to reduce the amount of mouse protein aimed first at eliminating the murine portion of constant regions of murine antibodies to form mouse/human chimerics. In clinical trials, chimeric immunoconjugates have markedly reduced anti-isotypic responses, but anti-idiotypic responses still occur (12–15). In an attempt to further reduce the immunogenicity of the antibody, humanized antibodies that incorporate human V region frameworks and C regions have been constructed. In this approach, only the CDRs[3] remain

[3] The abbreviations used are: CDR, complementarity determining region; CT, computed tomography; MX-DTPA, combined 1-p-isothiocyanatobenzyl 3-methyl- and 1-p-isothiocyanatobenzyl 4-methyl-diethylenetriamine pentaacetic acid; HPLC, high-performance liquid chromatography; SPECT, single-photon emission tomography; HAHA, human anti-humanized antibody.

Table 1 Patient clinical profiles

Patient	Age (yr)	BrE-3 serum antigen (µg/ml)	Previous treatment	Known disease
01HBR	52	0.89	CMF × 8 Tamoxifen Radiation-bone Megace	Bone, lung, liver
02HBR	61	0	FAC[a] × 6 Tamoxifen	Liver, lung, lymph nodes
03HBR	59	0.18	Tamoxifen Megace Lupron	Bone, liver, lymph nodes
04HBR	40	0	FAC (neoadjuvant)	Lung, lymph nodes
05HBR	58	0	Surgery only	Breast, lymph nodes
06HBR	33	Not available	FAC × 4 Tamoxifen	Bone, liver
07HBR	45	152	CMF × 6 FAC × 8 Navelbine	Chest wall, bone, lung

[a] FAC, 5-fluorouracil, Adriamycin, cytoxan.

from the original murine antibody. To eliminate or reduce the immunogenicity of BrE-3, its humanization was accomplished preserving the CDRs and using consensus human frameworks but achieving over 90% homology with human IgG1 sequences (16, 17). The measured affinity of the humanized antibody is almost three times that of the murine construct. When labeled with ^{90}Y, the humanized antibody shows equivalent efficacy in treatment of human breast carcinoma xenografts (16).

The purpose of this work was to study pharmacokinetics, toxicity, tumor targeting, and immunogenicity of ^{111}In-MX-DTPA humanized BrE-3 (huBrE-3) in the clinical setting. Based on these results and extrapolated absorbed dose estimates, a preliminary evaluation of the potential of MX-DTPA huBrE-3 labeled with ^{90}Y as a radioimmunotherapeutic agent could be made.

PATIENTS AND METHODS

Seven nonpregnant women, ages 33 to 61 years, with measurable or evaluable metastatic or recurrent breast cancer were studied (Table 1). All patients had localized disease at initial diagnosis of their primary. The interval between initial diagnosis and participation in the antibody study ranged from 15 months to 22 years. Each patient had tumor specimens obtained previously or current fine-needle aspirates of metastatic tumor that expressed the BrE-3 epitope in >25% of tumor cells by immunohistochemistry. All patients had normal renal and hepatic function, an adequate performance status (Karnofsky >60%), and no evidence of concurrent active collagen vascular disease or significant cardiac disease. No patient had a history of prior exposure to murine or human monoclonal antibodies. All patients were off cytotoxic therapy for at least 3 weeks before antibody infusion. Three patients were receiving hormone therapy at the time of radioimmunoconjugate administration. One patient was undergoing concurrent external beam radiotherapy to the spine. Each patient gave written informed consent as approved by the institutional review board. This study was performed under a United States Food and Drug Administration Investigational New Drug application sponsored by the Cancer Research Institute of Contra Costa.

Before administration of radioimmunoconjugate, a complete medical history was obtained, and a physical examination was performed. Complete blood counts with differentials and serum chemistries were obtained. Within 4 weeks of radioimmunoconjugate administration, chest radiographs, abdominal and pelvic CT scans, bone scans, and electrocardiograms were performed. CT scans were performed using i.v. contrast. Oral contrast was used for abdomen and pelvis studies. Slices, usually 10-mm thick on chest, abdomen, and pelvis CT and 7 mm through the liver, were contiguous. Areas of particular interest were studied with 7-mm slice thickness. Serum samples for measurement of circulating antigen reactive with humanized BrE-3 antibody and human anti-humanized BrE-3 antibody (HAHA) were also obtained at baseline.

Antibody. Humanized BrE-3 monoclonal antibody (huBrE-3) is an engineered immunoglobulin of human isotype IgG1 that has >90% humanized sequences (16). Only the CDR sequences remain from the original murine monoclonal antibody. The antibody reacts with an epitope on the M_r 400,000 breast epithelial mucin, which is a tandem repeat amino acid sequence (18). Its affinity for this epitope is approximately three times greater than the affinity of the murine BrE-3. The antibody was provided by the Cancer Research Institute of Contra Costa as a sterile, pyrogen-free solution and also conjugated to MX-DTPA, which is a mixture of 1-p-isothiocyanatobenzyl 3-methyl DTPA and 1-p-isothiocyanatobenzyl 4-methyl DTPA in PBS. The MX-DTPA was conjugated to huBrE-3, as reported previously by Brechbiel et al. (19). Briefly, huBrE-3 is dialyzed in the presence of 0.05 M HEPES buffer, pH 8.6, made up in 0.15 M sodium chloride, plus 1 g/l of Chelex 100 (Bio-Rad), overnight at 4°C. After this, huBrE-3 is placed in a ratio of 1:4 to MX-DTPA in the HEPES buffer plus sodium chloride and left for 19 h at room temperature. The conjugate is then dialyzed over 4 days in PBS, which is changed each day. The huBrE-3-MX-DTPA conjugate is then stored at 4°C. At this temperature, the antibody retains its original reactivity with antigen for up to 30 months thus far. The chelating activity has not decreased over this period of time. Stability in serum at 37°C for 1 week was demonstrated, with no loss of bound metal to chelate.

Radiolabeling. Approximately 6 mCi of pharmaceutical grade ^{111}In chloride (Mediphysics, Inc.) were buffered with 0.15 ·, sodium acetate and mixed with 2 mg of MX-DTPA huBrE-3 antibody for 20 min. Five mM sodium-calcium-EDTA was added to quench the reaction, and the reaction mixture was purified on a Sephadex G25 chromatography column. After purification, an average of 87 ± 8% of the total ^{111}In incubated with the MX-DTPA huBrE-3 was recovered as antibody-bound radioactivity. Instant TLC was developed in 5 mM EDTA on the pooled fractions containing radiolabeled antibody. The *Rf* of ^{111}In huBrE-3-MX-DTPA is practically 0, because the radiolabeled antibody remains at the origin. Radioactivity associated with immunoconjugate after purification was always >97%. The resultant product was diluted in 1% human serum albumin in normal saline, and aliquots were subjected to sterility testing and to a chromagenic assay to measure pyrogen levels, which never exceeded the upper limits of acceptability (5 EU/ml/kg). Immunoreactivity assessed by overnight incubation with antigen-coated beads was determined at an antibody concentration of 12.5 ng/ml. In all preparations, immunoreactivity was >68%.

Forty-eight mg of unconjugated huBrE-3 were diluted in 1% human serum albumin and mixed with the radiolabeled MX-DTPA huBrE-3. It was demonstrated previously that a coinjection of ~50 mg of unlabeled BrE-3 yielded optimal imaging and pharmacokinetic results (2, 3). A total volume of 250 ml was infused i.v. over 90 min. Vital signs were monitored during the infusion and for 1 h after completion of the infusion. At 72 h after infusion, repeat blood chemistries, complete blood counts, prothrombin time, and partial thromboplastin time were monitored.

Pharmacokinetics. Serial plasma samples were obtained at 5 min and 1, 2, 4, and 6 h and then at five succeeding time points through 8 days after completion of the infusion. Timed urine collections were performed through the eighth day after infusion. Plasma and urine samples were counted to measure total radioactivity. Plasma samples were further subjected to size exclusion, HPLC to identify antigen-antibody complexes, and intact antibody and breakdown products. Five hundred-μl samples were applied to a 10×600-mm BioSep SEC 3000 column equipped with a 10×50-mm guard column (Phenomenex, Torrance, CA). The mobile phase consisted of 0.05 M NaH_2PO_4, 0.1 M Na_2SO_4 buffer (pH 6.8) with an elution flow rate of 0.6 ml/min. Parallel detection was achieved with radiation detection followed by UV detection. Determination of radioactivity was performed using a BioScan Incorporated flow count detector (Bethesda, MD) equipped with an FC002 gamma flow cell. Data were processed at the 100-kilocount setting. A Perkin-Elmer LC-135 diode detector (Norwalk, CT) set at 280 nm was used for the UV detection. The 1-V output from both detector systems was interfaced to an Axxiom Chromatographic Data Controller system (Moorepark, CA). This permitted simultaneous plotting of the radioactive counts and UV absorbance. The column calibration was verified using a 20-μl injection of Bio-Rad gel filtration standard (thyroglobulin, bovine gamma globulin, chicken ovalbumin, equine myoglobin, and vitamin B_{12}). Blood clearance of radiolabeled antibody was determined. The half-time for clearance of the radiolabeled antibody was obtained using a nonlinear biexponential (two-compartment)

pharmacokinetic model. Initial parameter estimates were derived using the one of the three curve-stripping algorithms available within WINONLIN (Statistical Consultants, Apex, NC). These estimates were then applied to a biexponential to fit the time/concentration antibody data with respect to the duration of infusion and i.v. route of infusion. The key pharmacokinetic parameters obtained included half-lives for each compartment (*e.g.*, α and β) and the area under the concentration *versus* time curve expressed as a percentage of injected dose-hours.

Imaging and Image Analysis. Quantitative planar gamma camera imaging was performed to determine normal organ and tumor biodistribution, to identify tumor localization, and to make radiation absorbed dose estimates (20). All images were obtained with a digital large field of view gamma camera fitted with a medium energy collimator. Prior to radioimmunoconjugate administration, an ^{111}In transmission scan was acquired. Conjugate regional spot views or whole-body images were acquired at 4, 24, and 48–72 h and 7–8 days after infusion. Dual energy 20% windows centered on 173 and 247 keV, the energy windows of ^{111}In, were used. For whole-body imaging, scan speed was 5 cm/min. For regional spot imaging, 5-min acquisitions were performed for time points up to 72 h after injection and 7.5 min for more delayed images.

Whole-body counting was performed for those patients who underwent regional spot view imaging. For patients who underwent whole-body imaging, the whole-body images were used to obtain whole-body counts of radioactivity.

SPECT scans were obtained at the third (48–72 h) and fourth (7–8 day) imaging sessions. Either a triple-headed large field of view camera (Triad, Trionix Corp., Twinsburg, OH) acquired a total of 120 25-s projections, or a dual-headed camera (GCA7200; Toshiba Corp.) acquired a total of 90 40-s views over a 360° circumferential interval around the patient. Raw projection data were reconstructed into transaxial slices using filtered backprojection, and Chang's method of attenuation correction was applied. Resulting slices were 2 pixels thick (7.1 mm, Triad; 8.6 mm, GCA7200). Data were then reformatted into 2-pixel-thick coronal and sagittal slices.

Images were interpreted by a nuclear medicine physician with full knowledge of the extent of disease as assessed by physical examination and conventional imaging modalities. Only those lesions that showed increased uptake, even in the liver, were considered positive. Because this was an early Phase I study whose objective was to assess ^{111}In-MX-DTPA huBrE-3 localization in known tumors, blinded readings were not performed.

Using the planar images, region of interest and background templates were generated over normal organs and tumors for all imaging time points (21). Background subtraction, attenuation correction, and camera sensitivity derived from an ^{111}In standard of known activity were applied to all normal organ regions of interest to calculate the fraction injected activity (20, 22). Background subtraction and correction for camera sensitivity were applied to all tumor regions of interest. Attenuation correction was applied to the tumor regions when appropriate. The geometric mean of anterior and posterior activity was determined for large organs (*i.e.*, liver and lung). For smaller organs, *e.g.*, kidney, and for tumors the counts from a single planar projection (anterior and posterior) were used (20). Using the

Table 2 Average percentage of injected dose (%ID) ±SD at 4, 24, and 72 h and 8 days after injection and $T_{1/2\text{effective}}$ ($T_{1/2\text{eff}}$) for ^{111}In MX-DTPA huBrE-3 antibody

	%ID				$T_{1/2\text{eff}}$ (h)
	4 h	24 h	72 h	8 days	
Liver	16.7 ± 7.1%	19.7 ± 8.8%	19.3 ± 7.7%	18.7 ± 7.7%	63.6 ± 3.4
Spleen	1.8 ± 0.4%	1.9 ± 0.4%	1.7 ± 0.4%	1.7 ± 0.5%	58.5 ± 8.1
Kidney	3.7 ± 1.7%	4.4 ± 1.5%	4.4 ± 1.7%	4.1 ± 1.4%	50.4 ± 10.8
Lung	5.9 ± 2.4%	5.6 ± 1.9%	4.3 ± 1.7%	3.9 ± 1.7%	58.8 ± 6.1

fraction injected activity calculated for each time point, the biological and the effective half-lives of the radioimmunoconjugate were calculated for each normal organ and for measurable tumors that were visible on planar scans. A mono-exponential curve was assumed in all cases. Whole body remainder was calculated using both whole-body scans and urine clearance values, and the results were compared. The residence time was then calculated as follows:

$$\text{Residence time} = \text{Maximum fraction injected activity}$$

$$\times 1.44 \times T_{1/2\text{eff}}$$

Following the MIRD formulation and based on the ^{111}In-MX-DTPA huBrE-3 biodistribution, the radiation absorbed dose (per mCi or MBq administered) for the ^{111}In-labeled immunoconjugate was estimated using an implementation of the MIRDOSE program (23). By extrapolation from the ^{111}In biodistribution data to ^{90}Y-MX-DTPA huBrE-3, absorbed doses for the ^{90}Y-labeled immunoconjugate were estimated. Blood dose to marrow was calculated using the concentration of radioactivity in blood, assuming 25% of the marrow volume was blood and using the "S" factor for marrow dose from marrow (non-penetrating; Ref. 24). For ^{111}In, this calculated dose was added to the absorbed dose to marrow from other organs and the remainder of the whole body to determine red marrow absorbed dose (25). For ^{90}Y, the radiation absorbed dose from other organs and whole body were not included because the contribution from ^{90}Y in these sites to marrow constitutes <1% of the total marrow dose.

Radiation absorbed dose was calculated for at least one tumor that was both measurable on CT and identifiable on planar scintigraphy for each patient. The tumor size was determined by measurement of the tumor of interest in three planes on CT scans. Regions of interest were generated over these tumors on the sequential scans, and fraction injected activity and biological and effective half lives for radioactivity were calculated as described above. For tumors, a single planar projection was used to determine the activity within that region of interest. Tumor radiation absorbed doses from radioactivity accumulation in tumor was calculated by finding the volume based on the closest regular geometric shape, then calculating the radius of a sphere with the same volume and obtaining the absorbed fraction in water for a sphere of that radius (26).

HAHA. Serum samples for determination of HAHA were obtained at baseline, 8 days, 5 weeks, and 3 months after antibody infusion. HAHA response was assessed by incubating patients' serum with ^{125}I-labeled huBrE-3. After incubation, the mixture was run on an HPLC equipped with a guard column and two separation columns BioSep-SEC 3000 (Phenomenex) placed in series. Presence of HAHA was determined by detection of a decrease in transit time of a portion of the radiolabeled huBrE-3 representing the antibody-antibody immune. The level of this antibody-antibody complex was quantitated using a standard curve constructed by incubating 0.6–16 ng/ml of mouse anti-BrE-3 idiotype with the ^{125}I-labeled huBrE-3.

Immunohistochemistry. Immunohistochemical analysis of the expression of BrE-3 epitope in patient tumor samples was determined on formalin-fixed, paraffin-embedded tumor samples from breast and axillary lymph nodes and on cell smears derived from fine-needle aspiration of bone or visceral metastases. Immunohistochemical assays were performed using a modified avidin-biotin immunoperoxidase technique (Vectastain; Vector Laboratories, Burlingame, CA) with 3,3'-diaminobenzidine as a chromogen. Briefly, 5-μm tissue sections from surgical specimens were applied to poly-L-lysine-coated slides, and cell smears from fine-needle aspirates were prepared on coated slides, fixed in formalin for 5 min, and then held at −4°C in saline. Biotinylated humanized BrE-3, diluted to a final concentration of 25 μg/ml, was applied for 1 h to slides in an automated immunostainer (Cadenza; Shandon-Lipshaw, Pittsburgh, PA). A positive control (derived from formalin-fixed, paraffin-embedded sections of a breast carcinoma characterized previously) and a negative control (where primary antibody was substituted for serum) were run for each case.

Immunostained sections or cell smears were evaluated for positive immunoreactivity and scored from 1 to 4, depending upon intensity of staining. In addition, the percentage of immunoreactive tumor cells was determined and expressed in 10% increments. All seven patients demonstrated positive staining on immunohistochemistry of tumor tissue obtained previously or fine-needle aspirates.

RESULTS

Toxicity. No evidence of allergic reactions was observed among the seven patients evaluated in this study. One patient developed a grade 3 thrombocytopenia 9 days after administration of ^{111}In-MX-DTPA huBrE-3. Although this was in proximity to the administration of radioimmunoconjugate, it was felt that this thrombocytopenia could be attributed to the combination of concurrent external beam radiation to the spine and extensive bone marrow metastases as identified by bone scan and bone marrow biopsy. Analysis of blood failed to identify a serological basis for platelet destruction. No other significant alterations in blood chemistries or blood counts were identified.

Table 3   Whole-body and blood pharmacokinetics for ^{111}In-MX-DTPA huBrE-3 antibody

Patient	Whole-body $T_{1/2\text{eff}}$[a] (h)	Blood $T_{1/2\text{biol}}$[b]		Ag-Ab complex[c] (%)	AUC[d] (%ID × h)
		α (h)	β (h)		
01HBR	55.3		24.1	15.6	124.4
02HBR	61.5	23.3	100.7	2.9	211.2
03HBR	57.4	3.4	62.4	0.0	46
04HBR	60	16.8	192.6	0.0	309
05HBR	64	2.4	103.5	0.0	191
06HBR	62.1	1.6	119.3	0.0	487
07HBR	62.4	16.1	106.5	0.0	21
Mean ± SD	61.2 ± 2.1	10.6 ± 8.5	114.2 ± 39.2	2.6 ± 5.8	198.5 ± 139.6

[a] $T_{1/2\text{eff}}$, effective half life.
[b] $T_{1/2\text{biol}}$, biologic half life.
[c] Ag-Ab, antigen-antibody complex.
[d] AUC, area under the curve.

Biodistribution and Pharmacokinetics. The uptake of radioactivity at 4, 24, and 72 h and 8 days in normal organs is shown in Table 2. The average liver uptake over the course of imaging was ~20% of the injected dose of radioactivity. In fact, for all normal organs analyzed, the percentage of injected dose varied little over the course of the imaging. The mean effective half-life for the ^{111}In-MX-DTPA huBrE-3 for the whole body was 61.2 ± 2.1 h (Table 3).

The pharmacokinetics of radiolabeled antibody as determined by HPLC of plasma fit a biexponential model in six of seven patients. In these six patients, the $T_{1/2\alpha}$ averaged 10.6 ± 8.5 h, and the $T_{1/2\beta}$ averaged 114.2 ± 39.2 h. One patient (01HBR) demonstrated first-order kinetics with a $T_{1/2\text{bio}}$ of 24.1 h. This patient also demonstrated a relatively high percentage of radioactivity present as antigen-antibody complex (15.6%) in her plasma, although her baseline circulating antigen level was fairly low (Table 1). The average area under the curve for plasma radiolabeled antibody over time was 198.5 ± 139.6%ID·h for all seven patients.

Radiation Dose Estimates. For ^{111}In-MX-DTPA huBrE-3, the absorbed dose to the whole body averaged 0.53 ± 0.08 rads/mCi administered (Table 4). The average absorbed dose to the liver was 2.92 ± 1.40 rads/mCi. These estimates are comparable with that seen for the murine antibody. The biodistribution of ^{111}In-MX-DTPA huBrE-3 was used to extrapolate ^{90}Y-MX-DTPA huBrE-3 absorbed dose estimates. Whole-body absorbed doses averaged 2.46 ± 0.31 rads/mCi of ^{90}Y administered. This was similar to those calculated for the murine construct labeled with ^{90}Y (2, 3). However, again assuming that marrow irradiation came only from circulating radioimmunoconjugate, red marrow absorbed doses averaged higher (8.43 ± 11.88 rads/mCi) compared with the murine radioimmunoconjugate. This is at least in part due to the longer biological half-life of the humanized MX-DTPA BrE-3.

On the other hand, with one exception (patient 7 with 13 rads/mCi), we found consistently high tumor absorbed doses (43–101 rads/mCi). Tumor absorbed doses averaged 70.0 ± 31.5 rads/mCi of administered ^{90}Y. This gives an average tumor:marrow absorbed dose ratio of 8.3:1.

Imaging Results. Altogether, 105 sites of disease were identified by conventional diagnostic modalities including physical examination, bone scintigraphy, and CT. Of these, 76.2%

were identified on ^{111}In-MX-DTPA huBrE-3 whole-body and SPECT scans (Table 5). Sensitivities for lymph node metastases (88.9%) and lung metastases (84.6%) were the highest. Sensitivity for bone metastases was lower (71.9%). Five of 16 bony lesions in patient 6 were false negatives, possibly because many of these sites fell within the radiation therapy port. At the time of the antibody administration, this patient had already received a week of external beam radiation treatments. In other patients, bony lesions were obscured by underlying or nearby nonspecific uptake such as liver or blood pool. Among the 105 lesions, two sites, one lymph node and one bony, had not been identified previously. These were confirmed by repeat CTs (Fig. 1). Almost all liver lesions seen on antibody scan were identified by increased uptake (Fig. 2). The one exception was a cold lesion with a rim of mildly increased uptake, presumably representing necrosis surrounded by tumor. For most liver lesions, SPECT imaging was needed to identify the sites of abnormal uptake.

HAHA. Only one of the patients' serum showed detectable levels of HAHA. These very low levels were detected at 1 week (26.4 ng/ml) and 3 months (28 ng/ml) after antibody infusion in one patient. No other patients demonstrated levels of HAHA above the baseline average at any of the time points after huBrE-3 administration (8 days, 5 weeks, and 3 months).

DISCUSSION

In the seven patients whom we studied, ^{111}In-MX-DTPA huBrE-3 administration was tolerated extremely well. No allergic or antibody-related toxic reactions were identified. In the one patient who experienced a significant thrombocytopenia in temporal proximity to radioimmunoconjugate administration, no anti-platelet antibodies could be identified and, in fact, bone marrow examination revealed extensive tumor infiltration as the probable cause of her thrombocytopenia. As with the murine form of this antibody (2), we administered 50 mg to each patient without difficulty. Up to 500 mg of antibodies targeting similar antigens have been administered in earlier trials without complication (27). In the clinical trials of murine BrE-3, a few mild allergic reactions occurred (2, 3). Possibly, the humanization of the antibody may have reduced the likelihood of these reactions. This report provides still further evidence that toxicity from the antibody itself will not be problematic, possibly even with

Table 4 Radiation absorbed dose estimates based on biodistribution of ^{111}In-MX-DTPA huBRE-3 (rads/mCi administered)

	^{111}In-huBrE-3	^{90}Y-huBrE-3[a]
Kidneys	2.50 ± 0.71	23.99 ± 8.6
Liver	2.92 ± 1.40	21.10 ± 11.96
Lung	1.06 ± 0.26	8.99 ± 3.57
Ovaries	0.51 ± 0.08	1.55 ± 0.29
Red marrow[b]	0.95 ± 0.78	8.43 ± 11.88
Spleen	2.07 ± 0.70	19.59 ± 8.57
Urinary bladder	0.44 ± 0.15	1.23 ± 0.29
Whole body	0.53 ± 0.08	2.46 ± 0.31
Tumors		70.0 ± 31.5

[a] Extrapolated from ^{111}In-huBrE-3 biodistribution.

[b] Based on radioactivity in serial plasma samples and corrected for hematocrit.

Table 5 Imaging results

	True positives	False negatives	Total lesions	Sensitivity
Lymph nodes	8[a]	1	9	88.9
Liver	13	4	17	76.5
Lung	11	2	13	84.6
Bone	46[a]	18	64	71.9
Other	2		2	
Total	80	25	105	76.2

[a] Each of these categories includes one previously unsuspected and confirmed metastatic site.

repeated administrations. Immunogenicity may still be an issue in that setting, however.

Development of human anti-murine antibody has been a major obstacle to the clinical deployment of radioimmunotherapy with BrE-3 (3), preventing retreatment with the murine antibody in patients who developed an immune response even when clinical tumor responses were observed. Engineering of antibody proteins like humanized BrE-3 to reduce the foreign amino acid sequences aims to lessen, if not eliminate, the immunogenicity of the antibody.

Chimerization of other antibodies has proved effective in reducing the immunogenicity of these antibodies in some cases. For instance, chimeric 17-1A elicited very little immune response (12, 14). On the other hand, chimeric B72.3 elicited significant anti-chimeric immune responses in 7 of 12 patients (13) as did anti-CEA chimeric T84.66 in 2 of 3 patients (28). Chimeric NR-LU-13 elicited anti-chimeric immune responses in six of eight patients (15), albeit at a very low level.

Thus far, humanization of antibodies has reduced immunogenicity more consistently. Repeated dosing with humanized M195 elicited no measurable HAHA response in 13 patients, although 4 patients may have had very low-level responses (29). In a comparison of repeated dosing with murine anti-TAC antibody to a similar regimen with humanized anti-TAC in primates, an anti-humanized response was elicited later and at a lower level than the response to the murine anti-TAC regimen (30). With humanized BrE-3 antibody, only one patient showed any HAHA, and this very low level of HAHA may not have any clinical significance. This suggests that repeated dosing might be possible over a short interval without an alteration in pharmacokinetics. The incidence and magnitude of the HAHA response to BrE-3 may increase with repeated administrations. Both the influence of these low HAHA responses on pharmacokinetics and the effect of repeated administration will be examined in the upcoming dose-fractionation radioimmunotherapy trial.

Humanization of an antibody may be accompanied by reduced affinity and, *in vivo,* by changes in pharmacokinetics and biodistribution compared with the murine constructs. In the case of BrE-3 antibody, humanization was accomplished without loss of *in vitro* affinity for its antigen (16). By scintigraphy in our patients, we observed a slightly lower tumor localization rate (76%) than we did with the patients receiving the murine

BrE-3 (87%; Ref. 2). At least in part, this may have related to higher background ratios because humanized BrE-3 showed overall a longer half-time for clearance from blood than murine BrE-3. Persistent blood pool may have obscured uptake in some of the known lesions. It is less likely that this is related to antibody binding to tumor because fairly consistently high radiation doses were estimated for tumors in the group receiving humanized antibody (average, 70.0 ± 31.5 rads/mCi ^{90}Y administered; range for six tumors, 43–101 rads/mCi; one tumor, 13 rads/mCi). Estimated radiation absorbed dose in those tumors studied with the murine antibody ranged widely from ~2 rads/mCi administered to 590 rads/mCi in our earlier trial (2). The relatively high tumor doses found here are based only on imaging data. Biopsy after antibody administration was not available for validation of fraction injected dose estimates made from the planar images. Inaccuracies may be introduced when using image analysis for radiation absorbed dose because of unavailability of correct background measurements and inaccurate edge detection when generating regions of interest. Smaller tumor volumes may worsen these problems. The accuracy of this method is >10% for ^{131}I (20) but may be less for ^{111}In. For a 10-ml tumor, the reproducibility has been found to be 7.7% (20).

The pharmacokinetics of ^{111}In-MX-DTPA huBrE-3 appear to differ from the murine as well. The biodistribution in normal organs showed somewhat higher uptake in the liver than the murine construct. The average value for liver uptake was skewed somewhat by patient 1, who showed ~37% ID in the liver at 24 h. One explanation in this patient may be related to circulating antigen, although a higher level was observed in patient 7. The absolute amount of antigen measured was 0.89 μg/ml (Table 1), and HPLC showed 15.6% radioactivity present in plasma as antigen-antibody complex 5 min after infusion (Table 3). Clearance of antigen-antibody complex into the liver may have contributed to the high accumulation in liver. More likely, however, the high accumulation in the liver may have been due to occult but diffuse metastatic disease. Although this patient's liver CT was without evidence of tumor at the time of the antibody study, 2.5 months later, diffuse liver metastases were found. Possibly, some of the antibody uptake might be attributable to localization in diffuse microscopic metastases in this patient. The other three patients studied with liver metastases, patients 2, 3, and 6, also had relatively higher liver uptake (21.4, 24.3, and 20.8%, respectively) than the other patients because these metastases were included in the regions of interest used for analysis. The average percentage of uptake of the humanized radioimmunoconjugate in the spleen was slightly
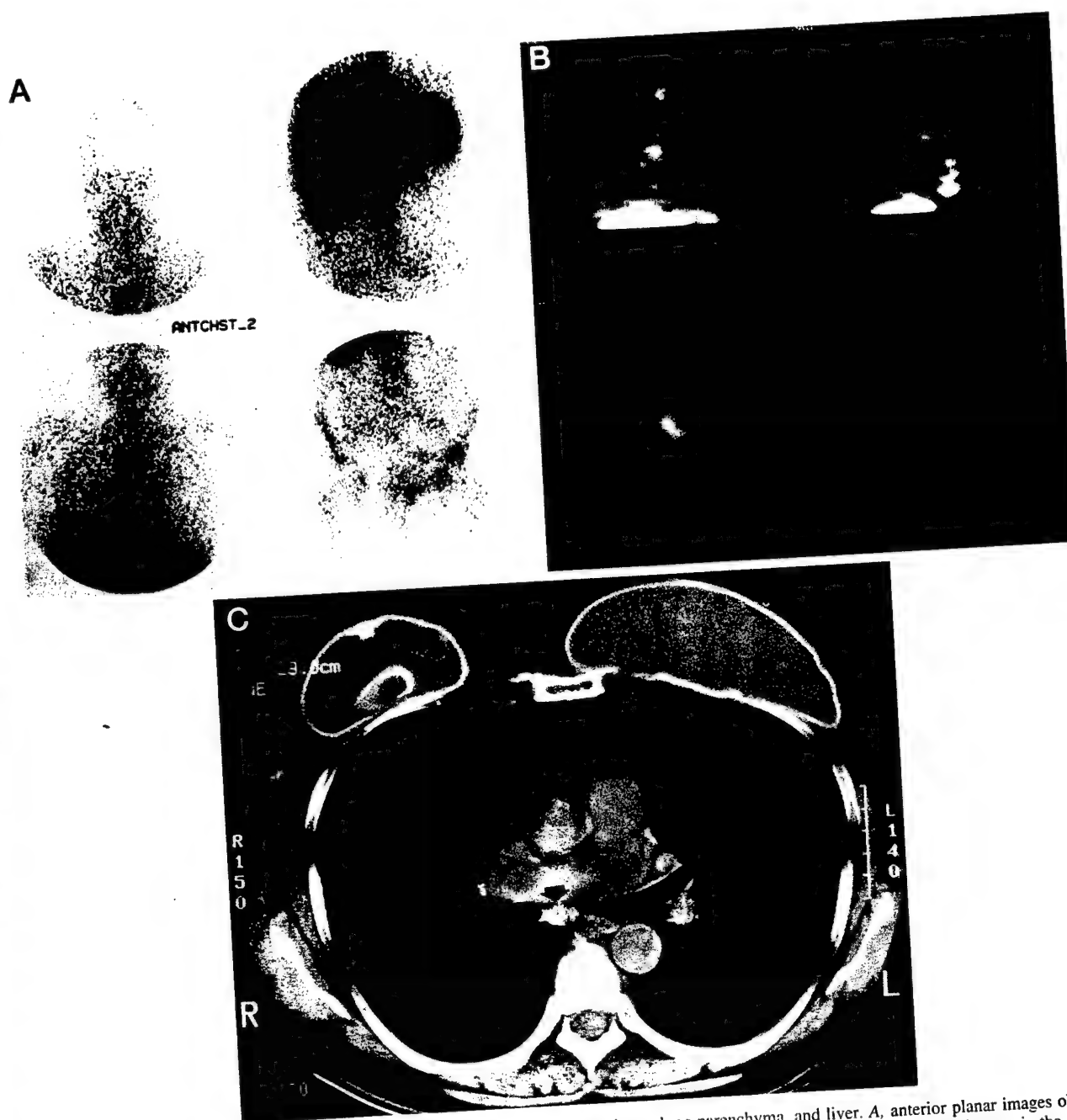
Fig. 1 A 52-year-old woman with known metastatic breast cancer to the skeleton, lung parenchyma, and liver. A, anterior planar images obtained 72 h after administration of 5 mCi of ^{111}In-MX-DTPA huBrE-3 suggests focal uptake in the mediastinum (arrow). B, SPECT images in the coronal (top left), sagittal (top right), and transaxial (bottom left) planes show this focus of uptake more clearly in the mediastinum at approximately the level of the carina and slightly to the right of midline. C, a transaxial slice from the chest CT obtained a week prior to the antibody study shows a borderline enlarged (1 cm) lymph node in the subcarinal region slightly to the right of midline (arrow). This was recognized only on retrospective review of the CT.

higher in the seven patients studied compared with patients receiving murine BrE-3. Compared with the normal organ bio-distribution of the murine radioimmunoconjugate, the values for the humanized radioimmunoconjugate showed little decrease over the first week. This may be related to the longer blood clearance observed for the humanized construct.

The half time for blood clearance for humanized BrE-3 ($T_{1/2bio\beta} = 114.2 \pm 39.2$) averaged slightly more than twice as long as the blood clearance half-time for the murine antibody ($T_{1/2bio\beta} = 56 \pm 25.4$; Ref. 2). Prolonged half-time for blood clearance has been reported for some other humanized antibodies (30) and for chimeric B72.3 (13, 31), NR-LU-13 (15), and 17-1A (12). Longer half-times may translate into prolonged access to tumor for the radioimmunoconjugate. On the other
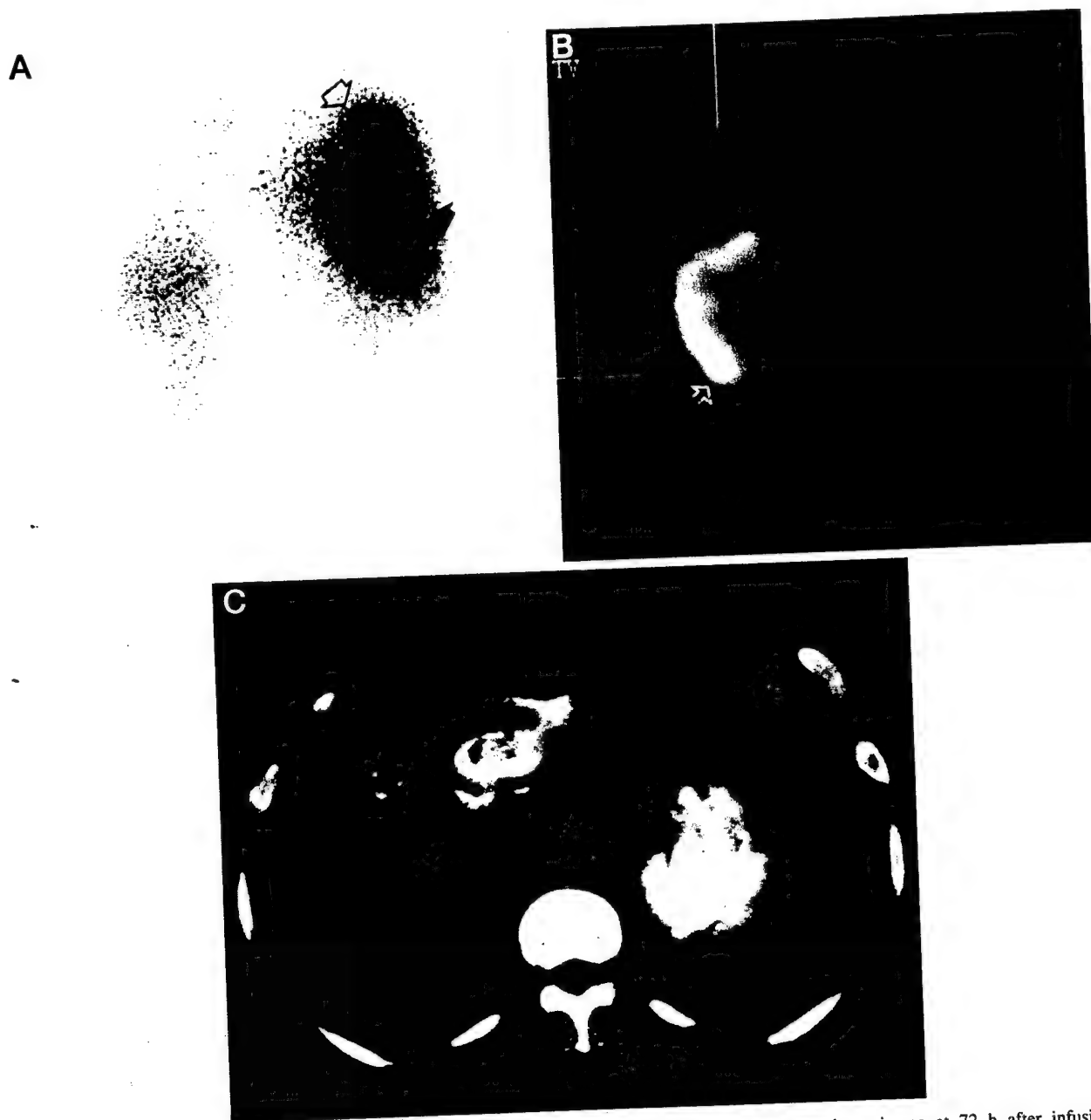
Fig. 2 A 61-year-old woman with progression of disease while on Tamoxifen. In A, a posterior planar image at 72 h after infusion of ^{111}In-MX-DTPA huBrE-3 shows a distinct focus of uptake at the top of the liver, which corresponded to a lung metastasis at the right base (*open arrow*) and a less well-defined area of increased uptake in the mid portion of the right lobe (*arrow*). In B, a transaxial SPECT slice through the liver metastasis shows this increased uptake in the posterior aspect of the right lobe more distinctly (*open arrow*). In C, CT slice at the same level obtained at the time of the antibody scan shows multiple hypodense liver metastases, the largest one of which corresponds to the abnormal uptake within the liver.

hand, as with these other antibodies, the prolonged half-time in blood for huBrE-3 translates into a higher radiation absorbed dose to marrow. Marrow absorbed dose estimates for ^{90}Y-labeled huBrE-3 in six patients ranged from 1.9 to 9.9 rads/mCi ^{90}Y administered. In one patient, the absorbed dose estimated was 34.5 rads/mCi [90]Y. In our patients, an average tumor:marrow absorbed dose ratio for ^{90}Y-MX-DTPA huBrE-3 of 8.3:1 was found. However, the individual tumor:marrow ratios ranged from 2.0 to 50.2. These ratios, which are an indication of therapeutic index, may be sufficient to achieve tumor responses in some patients.

The variability in tumor and marrow absorbed dose estimates in our patients raises a further point about the use of radioimmunolocalization in radioimmunotherapy. Because ^{90}Y is difficult to image, we have chosen to coadminister ^{111}In-labeled antibody to trace normal organ and tumor uptake. In the

setting of our Phase I trial, where pharmacokinetics and biodistribution are important end points, this has been the only means of estimating the behavior of the therapeutically labeled (^{90}Y) immunoconjugate. However, in the setting of more routine clinical therapy, pretherapy imaging may be a sounder strategy to predict which patients' tumors will be targeted by radioimmunoconjugate and in which patients excessive normal organ radiation absorbed dose would make radioimmunotherapy unacceptably toxic.

The utility of the latter approach will depend on our ability to use ^{111}In radioimmunoconjugate to predict the behavior of ^{90}Y radioimmunoconjugate. It is recognized that free ^{111}In and ^{90}Y will behave differently *in vivo*, with ^{90}Y seeking bone mineral and ^{111}In binding to marrow-associated proteins. It is also expected that the stability of the ^{111}In conjugate bonds may differ from that of ^{90}Y conjugates. The validity of using ^{111}In to predict ^{90}Y radiation absorbed dose will also depend on the reproducibility of the behavior of radioimmunoconjugate between two closely spaced administrations. Analysis of blood and urine pharmacokinetics for ^{90}Y-MX-DTPA huBrE-3 and ^{111}In-MX-DTPA huBrE-3 when they are coadministered in the context of the ongoing dose fractionation therapy trial will answer some of these questions.

In conclusion, this initial radioimmunolocalization trial has shown that the engineered humanized construct of BrE-3 antibody is much less immunogenic than the murine form. Tumor localization is comparable, although background clearance is slower. The prolonged blood clearance appears to have implications for increasing red marrow absorbed dose. Although this is not a serious consideration for antibody labeled with a diagnostic amount of ^{111}In, this may decrease the therapeutic index of ^{90}Y-labeled huBrE-3. Initial estimates of tumor absorbed dose for ^{90}Y-labeled huBrE-3 give an average tumor:marrow ratio of 8.3 with a fair degree of interpatient variability. We anticipate that the reduced immunogenicity of humanized BrE-3 will permit repeated administration and improve the possibility of prolonged therapy with ^{90}Y-labeled huBrE-3. Strategies in radioimmunotherapy that may offer some marrow protection like dose fractionation should be feasible with this reduced immunogenicity. The efficacy of such an approach is now being tested in a radioimmunotherapy trial. However, it may be necessary to incorporate other types of marrow support to achieve tumoricidal effects. Alternatively, strategies to enhance the radiation effect at the tumor site may prove effective.

ACKNOWLEDGMENTS

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INITIAL STUDIES IN PATIENTS WITH ADVANCED BREAST CANCER USING IN-111 MX-DTPA HUMANIZED BRE-3 (HUBRE-3) MONOCLONAL ANTIBODY.

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To evaluate radiolabeled MX-DTPA huBrE-3 as a radioimmunotherapeutic agent in breast cancer, preliminary imaging, pharmacokinetic, and dosimetry studies with In-111 MX-DTPA huBrE-3 have been performed in patients with metastatic breast cancer expressing BrE-3 antigen. To date two patients have been studied. Patients received 2 mg MX-DTPA huBrE-3 labeled with 3-4 mCi In-111 combined with 48 mg of nonconjugated huBrE-3 over an hour. Serial blood samples, gamma camera imaging, whole body counting, and continuous urine collection were performed over one week. Abnormal sites of uptake on scan were compared to known lesions documented by conventional methods. Pharmacokinetic modeling of plasma samples which were subjected to HPLC was performed. Radiation dose estimates for normal organs and measurable tumors were made using the standard MIRD formalism. Immunogenicity of the huBrE-3 was evaluated up to 3 months after administration.

No significant toxicity or allergic reactions were observed. Twenty-two of 26 known sites of disease were imaged on planar and or SPECT scans. Two previously unsuspected sites of disease were seen and confirmed. Overall sensitivity was 86%. In one patient, mono-exponential modeling yielded a plasma T1/2 of 24.05 ±4.5 hrs and in the second a bi-exponential model gave an $T1/2_{alpha}=23.3\pm258.5$ hrs and $T1/2_{beta}= 100.7 \pm793.8$ hrs. AUC's were 1.91 and 6.32 mCi·hrs, respectively. Average whole body dose for In-111 huBrE-3 was 0.55±0.10 rads/mCi. Whole body dose extrapolated to Y-90 huBrE-3 averaged 2.55±0.39 rads/mCi. Tumor doses averaged 95.5±8.3 rads/mCi. Serum samples at 5 weeks and 3 months after huBrE-3 administration showed only trace amounts of reactivity with both huBrE-3 and murine BrE-3.

The excellent tumor localization, favorable pharmacokinetics, and initial radiation dose estimates support the further evaluation of radiolabeled MX-DTPA huBrE-3 as a potential therapeutic agent. Although slightly immunogenic, the levels observed should not preclude repeated administrations. Further studies are anticipated.

LOCALIZATION OF IN-111 MX-DTPA HUMANIZED BRE-3 MAB IN PATIENTS WITH ADVANCED BREAST CANCER.

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To evaluate MX-DTPA humanized BrE-3v1 monoclonal antibody (huBrE-3) as a potential radiotherapeutic immunoconjugate in breast cancer, In-111 MX-DTPA huBrE-3(2 mg, 185 MBq) was administered i.v. with 48 mg of nonconjugated huBrE-3 to patients with metastatic breast cancer. Serial whole body counting, gamma camera imaging, plasma, and urine sampling were performed to assess pharmacokinetics, radiation dose, and tumor localization. Scan results were compared to sites of tumor documented by conventional modalities. Pharmacokinetic modeling of plasma radioactivity and radiolabeled antibody was performed. Radiation dose was estimated using standard MIRD formalism. Two patients with 26 metastatic sites total have been studied so far. No toxicity or allergic reactions have been observed. 22/26 (84.6%) sites were identified on scans including liver, lung and bone metastases. Two previously unknown sites are suspected. Plasma T1/2 clearances for radiolabeled antibody averaged 45.7 ±28.1 hrs. AUC averaged 1.55± 1.02 %ID-hrs. A shorter plasma T1/2 and smaller AUC were associated with higher circulating antigen. Percent radioactivity excreted in urine averaged 71±22% in the first 48 hours. Calculated whole body dose for In-111 was 0.17 mGy/MBq. Percent ID/g estimated from serial imaging was 0.0004% in a measurable bone tumor. Extrapolating In-111 biodistribution to Y-90 biodistribution and radiation dosimetry, we estimate a dose to this tumor of 20mGy/MBq administered Y-90-huBrE-3 with a 0.42 mGy/MBq dose to marrow.

These early results demonstrate favorable pharmacokinetics and suggest that Y-90 huBrE-3 may be an active radioimmunotherapeutic agent. Immunogenicity studies and further patient studies are proceeding. (Supported by NIH RO1 CA61455)

rats given BSH and BPA i.c. with BBB-D was 73 d. ~~with~~ ~~survivors~~ (LTS), compared to 65 d. without ~~BPA~~ ~~d. for i.v. injected rats,~~ and 31 d. for irradia~~t~~ ~~results~~ indicate that i.c. injection of BSH and BPA with ~~BBB-D resulted in~~ a significant enhancement in survival time compared ~~with the regimen~~ used to treat this tumor.

#1658 Inhibition by gallotannins and proanthocyanidins of the biochemical markers of skin tumor promotion by Ultraviolet B radiation. Gali-Muhtasib, H.U., Khatib, S., Perchellet, J.P., and Samad, A. American University of Beirut, Biology Division, Beirut, Lebanon, Kansas State University, Biology, Manhattan, KS 66506

Topical applications of hydrolyzable (HTs) and condensed tannins (CTs) were tested in SKH-1 hairless mice for their ability to inhibit the stimulations of ornithine decarboxylase (ODC) activity and DNA synthesis linked to skin tumor promotion by Ultraviolet B (UVB) radiation. The peak of ODC induction observed 30h after a single UVB radiation at 100 mJ/cm^2 shifts to an earlier time of 5h after 3 UVB treatments repeated at 24h interval. ODC activity is even induced 1h after such multiple UVB treatment. Similarly, the peak of UVB-stimulated DNA synthesis observed 48h after a single dose of 125mJ/cm^2 shifts to 16h after 3 UVB treatments at 24h interval. When applied 10min before each UVB treatment, 34mg of HTs or CTs inhibit the ODC and DNA responses respectively observed either 30h and 40h after a single UVB (200mJ/cm^2) or 5h and 16h after 3 UVB treatments (100mJ/cm^2) at 24h interval by at least 45–75%. Commercial tannic acid (TA) and Tarapod TA, the two most effective among the HTs and CTs tested, inhibit the ODC and DNA responses to UVB to a greater degree than equal doses of their respective monomeric units, gallic acid and catechin. Thus, oligomeric HTs and CTs inhibit the skin tumor-promoting activity by UVB radiation. Supported by the American University of Beirut Research Board, the Lebanese Research Council and Bioserve Space Technologies NAGW-1197.

#1659 Synergistic activity of radioimmunotherapy with prolonged Topotecan infusion in human breast cancer xenograft. Ng, B., Liebes, L., Kramer, E., Wasserheit, C., Hochsler, H., Blank, E., Ceriani, R., and Furmanski, P. New York University, New York, NY 10003

The topoisomerase I inhibitors are a unique class of chemotherapeutic agents that act to interfere with DNA breakage-reunion by inhibiting the action of topoisomerase I. Prolonged infusion of Topotecan may improve activity of radiation damage. This study examined the effect of radioimmunotherapy with Topotecan on human breast cancer growth in vivo. The radio-immunoconjugate used is ^{90}Y MX-DPTA BrE-3 monoclonal antibody which recognizes an epitope of breast epithelial mucin expressed in most breast cancers. Nude mice were implanted with human mammary carcinoma (MX-1). On day 21, mice were randomized into 4 groups: CTL (no treatment), BrE-3 (i.p. 50ug of BrE3 labeled with 200uCi of ^{90}Y), Topo (Topotecan 1 mg/kg for 14 days via s.c. Alzet pump), and Combo (BrE-3 & Topo). Body weights, tumor weights and mortality were recorded for 60 days. Data expressed as mean ± SD, statistical analysis by one-way ANOVA except for mortality which was by log-rank.

| Group | n | Body Weights (gm) | Tumor Weights (mg) | | 60 days Mortality |
			45 days	60 days	
CTL	6	23 ± 2	632 ± 45	850 ± 52	100
BrE-3	7	22 ± 1	316 ± 38†	759 ± 48	85
Topo	7	23 ± 2	281 ± 35†	780 ± 58	85
Combo	7	24 ± 2	35 ± 8*	45 ± 10*	15*

† p < 0.05 vs. CTL, * p < 0.05 vs. BrE-3 and Topo

Topotecan or BrE-3 treatment only delayed the overall tumor growth rate up to 45 days but did not affect survival. The combination of radioimmunotherapy with Topotecan substantially reduced tumor growth and prolonged tumor-free survival in a synergistic fashion. Preliminary in vitro studies demonstrated increased cell kill with this combination as well.

#1660 Racial differences in breast fibrosis following lumpectomy and radiotherapy. Demouy, J., Person, K., Ampil, F., and Burton, G. Louisiana State University Medical Center, Shreveport, LA 71130

Breast conservation with lumpectomy and radiation is effective therapy for early stage breast cancer, but some patients suffer significant radiation-induced breast fibrosis. We have developed an objective system to evaluate breast fibrosis. Patient population: Women (49) who had completed lumpectomy and radiation therapy >6 months prior to evaluation. Methods: Patients were interviewed to obtain demographic data. A visual scale was used to record patients' breast pain and satisfaction. Breast volumes were determined. Bilateral breast density was measured using a "densitometer" over the nipple and in each breast quadrant. The densitometer, a weighted plunger (100 gm/cm^2), compressed the breast with the depth of compression measured in centimeters. The lumpectomy site was censored from analysis. Breast compression in cm was totaled and compared to the contralateral breast. Results: All patients were satisfied with their breast cancer treatment. Breast density (fibrosis) was increased in African American as compared to Caucasian women (M = 1.85 cm [range 0.2 - 3.8] vs M = 1.0 cm

[range 0.3 - 2.2] p < 0.01), but was not associated with breast size, age, menopausal status, current or prior history of smoking, diabetes or patient satisfaction. Conclusion: Significant breast fibrosis is an uncommon complication. Patients are satisfied with the cosmetic results despite significant fibrosis. African American women regardless have a statistically significant increased risk of breast fibrosis. This risk should not deter breast conservation as patient satisfaction is overwhelmingly favorable.

#1661 Influence of fractionated x-irradiation therapy in the assessment of normal tissue response. Vincent, P.W., Elliott, W.L., Howard, C.T., Leopold, W.R., Lotarski, S.M., Parker, R.J., and Roberts, B.J. Parke-Davis Pharm Res. Div. of Warner-Lambert Co., Ann Arbor, MI 48105

X-irradiation induced leg contracture in rats was used to assess the correlation of local tissue response between single dose and fractionated radiation therapy. Significant dose dependent contractures in leg extendibility were correlated with clinical and pathological findings within 4 weeks of receiving single doses of 20 and 40 Gy. Animals receiving identical total doses of irradiation over 5 days exhibited a lesser degree of leg contracture that was not apparent until 11 weeks post therapy. To investigate the possibility of increased localized toxicity from protracted combination therapy in normal tissue, animals were administered solutions of a hypoxic cell cytotoxin (CI-1010) IV and 2.5 Gy irradiation on a clinically relevant treatment schedule, 5 days per week for 7 weeks. Nine weeks after treatment, mild leg contracture was observed only in the irradiated control animals. Daily administration of CI-1010 for 7 weeks IV in combination with localized irradiation did not produce enhanced local tissue damage over that obtained with either treatment alone. The degree of leg contracture in rats correlated with single doses of irradiation, but there was no apparent correlation when a clinically relevant treatment protocol was used. These data indicate that leg contracture as an assessment of normal tissue response is highly dependent on the treatment schedule used.

#1662 T ICAM-1 expression in the pulmonary vascular endothelium in response to thoracic irradiation. Hallahan, D., Subbulakshmi, V., and Kuchibhotla, J. Department of Radiation and Cellular Oncology, University of Chicago and Pritzker School of Medicine, Chicago, IL 60637

We studied expression of cell adhesion molecules (CAMs) in lungs from mice treated with thoracic irradiation to determine the mechanisms by which ionizing radiation induces pathogenic inflammatory response in the lung. After x-irradiation, intercellular adhesion molecule-1 (ICAM-1) was expressed in pulmonary capillary endothelial cells and not in the endothelium of large vessels, whereas E-selectin was expressed in the endothelium of both large vessels and capillaries within the lung. This pattern of CAM expression was observed in all three mouse strains studied (C3H, C57/B6, and nude mice). Anti-ICAM-1 blocking antibodies attenuated leukocyte binding to irradiated endothelial cells in culture. Moreover, anti-ICAM-1 blocking antibodies injected into the tail veins of mice following thoracic irradiation reduced the number of inflammatory cells observed within the irradiated lung. ICAM-1 was not expressed in the lungs of ICAM-1-deficient mice treated with thoracic irradiation, and there was no leukocyte infiltration into their lungs. These data demonstrate that the cell adhesion molecules ICAM-1 and E-selectin are expressed in pulmonary endothelial cells following thoracic irradiation of mice and that ICAM-1 participates in inflammatory cell infiltration into the irradiated lung.

#1663 Quantitative immunohistochemical analysis of hypoxia, proliferation and vascular endothelial growth factor in human tumors. Raleigh, J.A., Kennedy, A.S., Rinker, L.H., Calkins, D.P., Novotny, D.B., Fowler, W.C., Weissler, M.C., and Varia, M.A. Radiation Oncology, UNC School of Medicine, Chapel Hill, NC 27599

The purpose of this clinical study is to quantitate hypoxia, proliferation and vascular endothelial growth factor (VEGF) in human tumors. We believe that these factors are interrelated and that, while hypoxia alone has some predictive value, combined hypoxia, proliferation and VEGF data will provide for a more accurate predictor of therapeutic outcome—not only for radiotherapy but also for chemotherapy and surgery. A novel immunoperoxidase technique based on pimonidazole binding was used to measure tumor hypoxia in formalin-fixed, paraffin-embedded biopsy samples. Commercially available antibodies were used for immunohistochemical analyses of proliferating cell nuclear antigen (PCNA) and VEGF on tissue sections contiguous to those immunostained for pimonidazole binding. Eleven patients have been accrued—9 with uterine cervix tumors and 2 with head and neck tumors. Qualitatively, all possess hypoxia and there is little or no overlap between areas of hypoxia and proliferation. Quantitation based on Chalkley point counting of immunostained cells in 3 to 4 biopsies from each tumor has provided preliminary data that indicate that hypoxia is not related to FIGO stage; is inversely related to proliferation; and, is directly related to VEGF expression at low and intermediate, but not high, levels of tumor hypoxia. We continue to accrue patients. An updated report of both qualitative and quantitative data for all patients will be presented. Supported by DHHS NCI R01-CA50995 and R42-CA68826 and by the Lineberger Comprehensive Cancer Center.